SEVERN TRENT SERVICES

STL SACRAMENTO STANDARD OPERATING PROCEDURE
TITLE: METHOD 8290 - POLYCHLORINATED DIOXINS & FURANS BY HRGC/HRMS

(SUPERSEDES: SAC-ID-0005, REVISION 4.0, 9/11/98)

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1. SCOPE AND APPLICATION

1.1. This method provides procedures for the detection and quantitative measurement of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), polychlorinated dibenzo-p-dioxins (tetra- through octachlorinated homologs; PCDDs), and polychlorinated dibenzofurans (tetra- through octachlorinated homologs; PCDFs) in a variety of environmental matrices at part-per-trillion (ppt) concentrations by SW 846 Method 8290. The analytical method calls for the use of high-resolution gas chromatography and high-resolution mass spectrometry (HRGC/HRMS) on purified sample extracts. An optional method for reporting the analytical results using a 2,3,7,8-TCDD toxicity equivalency factor (TEF) is also described. Table 1 lists the various sample types covered by this analytical protocol, the 2,3,7,8-TCDD-based method calibration limits and other pertinent information.

1.2. The sensitivity of this method is dependent upon the level of interferences within a given matrix.

1.3. This method is designed for use by analysts who are experienced with residue analysis and skilled in high-resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS).

1.4. Samples containing concentrations of specific congeners (PCDDs and PCDFs) that are greater than the calibration limit should be analyzed by a protocol designed for such concentrations, such as 8280A.

2. SUMMARY OF METHOD

2.1. This procedure uses matrix-specific extraction, analyte-specific cleanup, and high resolution capillary column gas chromatography/high resolution mass spectrometry (HRGC/HRMS) techniques.

2.2. If interferences are encountered, the method provides selected cleanup procedures to aid the analyst in their elimination. A simplified analysis flow chart is show in Figure 1.

2.3. A specified amount (see Table 1) of soil, sediment, fly ash, water, sludge (including paper pulp), still-bottom, fuel oil, chemical reactor residue, air sample (QFF, PUF or XAD media) or fish tissue, is spiked with a solution containing specified amounts of each of nine isotopically (^{13}C) labeled PCDDs/PCDFs listed in Table 2. The sample is then extracted according to a matrix-specified extraction procedure. The extraction procedures are: a) toluene Soxhlet extraction for soil, sediment, fly ash samples, aqueous sludges, and solid air matrices (XAD, QFF, PUF); b) methylene chloride liquid-liquid extraction for water samples; c) dilution of a

small sample aliquot in solvent for wastes/chemical products; and d) toluene (or hexane/methylene chloride) Soxhlet extraction for fish tissue.

2.4. If interferences are present, extracts may be cleaned as described below. The extracts are submitted to an acid and/or base washing treatment and dried. Following a solvent exchange step, the residue is cleaned up by column chromatography on acid/base silica, acid alumina and carbon on silica. The preparation of the final extract for HRGC/HRMS analysis is accomplished by adding 20 µL (depending on the matrix type) of a tetradecane solution containing 100 pg/µL of each of the two recovery standards 13C-1,2,3,4-TCDD and 13C-1,2,3,7,8,9-HxCDD (Table 2) to the concentrated eluate. The former is used to determine the percent recoveries of tetra- and penta-chlorinated PCDD/PCDF internal standards while the latter is used for the determination of hexa-, hepta- and octa-chlorinated PCDD/PCDF internal standard percent recoveries.

2.5. One to two µL of the concentrated extract are injected into an HRGC/HRMS system capable of performing selected ion monitoring at resolving powers of at least 10,000 (10 percent valley definition).

2.6. The identification of ten of the 2,3,7,8-substituted congeners (Table 3), for which a 13C-labeled standard is included as a spiked compound, is based on their elution at their exact retention time (-1 to +3 seconds from the respective internal or recovery standard signal) and simultaneous detection of the two most abundant ions in the molecular ion region. All other identified PCDD/PCDF congeners are identified by their relative retention times based on the daily CCV standard , and the simultaneous detection of the two most abundant ions in the molecular ion region. Confirmation is based on a comparison of the ratio of the integrated ion abundance of the molecular ion species to their theoretical abundance ratio.

2.7. Quantification of the individual congeners, total PCDDs and total PCDFs is achieved in conjunction with the establishment of a multipoint (five points) calibration curve for each homolog, during which each calibration solution is analyzed once.

3. DEFINITIONS

3.1. Definitions of terms used in this SOP may be found in the glossary of the Laboratory Quality Manual (LQM).

3.2. Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs): compounds (Figure 2) that contain from one to eight chlorine atoms. The seventeen 2,3,7,8-

substituted PCDDs and PCDFs are shown in Table 3. The number of isomers at different chlorination levels is shown in Table 4.

3.3. Homologous series: Defined as a group of chlorinated dibenzodioxins or dibenzofurans having a specific number of chlorine atoms.

3.4. Isomer: Defined by the arrangement of chlorine atoms within an homologous series. For example, 2,3,7,8-TCDD is a TCDD isomer.

3.5. Congener: Any isomer of any homologous series.

3.6. Surrogate Standards: A 13C-labeled analog or mixture of analogs that are added to the sample collection media prior to shipment toe the field.

3.7. Internal Standard: An internal standard is a 13C-labeled analog of a congener chosen from the compounds listed in Table 3. Internal standards are added to all samples including method blanks and quality control samples before extraction, and they are used to quantitate the concentration of the analytes. Nine internal standards are used in this method. There is one for each of the dioxin and furan homologs (except for OCDF) with the degree of chlorination ranging from four to eight. Additional internal standards may be added to act as retention time references, but they are not used for quantitation.

3.8. Recovery Standard: Two recovery standards are used to determine the percent recoveries for the internal standards. The 13C-1,2,3,4-TCDD is used to measure the percent recoveries of the tetra- and pentachlorinated internal standards while 13C-1,2,3,7,8,9-HxCDD is used to determine the recovery of the hexa-hepta- and octachlorinated internal standards . 13C-1,2,3,7,8,9-HxCDD also acts as a retention time reference for the unlabeled analog present in sample extracts. They are added to the final sample extract before HRGC/HRMS instrument analysis.

3.9. High-Resolution Concentration Calibration Solutions (Table 5): Tetradecane solutions containing known amounts of the 17 2,3,7,8-substituted PCDDs and PCDFs, a minimum of nine internal standards (13C-labeled PCDDs/PCDFs), and two carbon-labeled recovery standards; the set of five solutions is used to determine the instrument response of the unlabeled analytes relative to the internal standards and of the internal standards relative to the recovery standards.

3.10. Sample Fortification Solution (Table 2): A solution (isooctane or toluene) containing the nine internal standards, which is used to spike all samples before extraction and cleanup.

3.11. Recovery Standard Solution (Table 2): A tetradecane solution containing the two recovery standards, which is added to the final sample extract before HRGC/HRMS analysis.

3.12. Field Blank: A portion of a sample representative of the matrix under consideration, which is used to assess potential PCDD/F contribution from sampling and transport.

3.13. Rinsate: A portion of solvent used to rinse sampling equipment. The rinsate is analyzed to demonstrate that samples were not contaminated during sampling.

3.14. GC Column Performance Check Mixture: A tetradecane solution containing a mixture of selected PCDD/PCDF standards including the first and last eluters for each homologous series, which is used to demonstrate continued acceptable performance of the capillary column (i.e., ≤ 25 percent valley separation of 2,3,7,8-TCDD from all the other 21 TCDD isomers) and to define the homologous PCDD/PCDF retention time windows.

3.15. Performance Evaluation Materials (PEM's): Representative sample portions containing known amounts of certain unlabeled PCDD/PCDF congeners (in particular the ones having a 2,3,7,8-substitution pattern). Representative interferences may be present. PEMs may be obtained from the EPA EMSL-LV or other sources and submitted to potential contract laboratories, which must analyze these and obtain acceptable results before being awarded a contract for sample analyses (see IFB Pre-Award Bid Confirmations). PEMs may also be included as unspecified ("blind") quality control (QC) samples in any sample batch submitted to a laboratory for analysis.

3.16. Relative Response Factor: Response of the mass spectrometer to a known amount of a native analyte relative to a known amount of an internal standard, or a known amount of internal standard to a known amount of a recovery standard.

3.17. Sample Re-extraction: Extraction of another portion of the sample followed by extract cleanup and extract analysis.

3.18. Extract Reanalysis: Instrument analysis by HRGC/HRMS of another aliquot of the final extract.

3.19. Tuning (Mass Resolution Check): Standard method used to demonstrate a static resolving power of 10,000 minimum (10 percent valley definition).

3.20. Method Calibration Limits: For a given sample size, a final extract volume, and the lowest and highest concentration calibration solutions, the lower and upper calibration limits delineate the region of quantification for which the HRGC/HRMS system was calibrated with standard.

3.21. Matrix Spike Fortification Solution: Solution used to prepare the laboratory control sample, matrix spike, and matrix spike duplicate samples. It contains all unlabeled analytes listed in Table 5. The solution also contains all internal standards used in the sample fortification solution per the method

4. INTERFERENCES

4.1. Solvents, reagents, glassware and other sample processing hardware may yield discrete artifacts or elevated baselines that may cause misinterpretation of the chromatographic data. All of these materials must be demonstrated to be free from interferents under the conditions of analysis by running laboratory method blanks. Analysts shall not use PVC gloves.

4.2. The use of high-purity reagents and solvents helps minimize interference problems. Purification of solvents by distillation in all-glass systems may be necessary.

4.3. Re-use of glassware is to be minimized to avoid the risk of contamination.

4.4. Interferents co-extracted from the sample will vary considerably from matrix to matrix. PCDDs and PCDFs are often associated with other interfering chlorinated substances such as polychlorinated biphenyls (PCBs), polychlorinated diphenyl ethers (PCDPEs), polychlorinated naphthalenes, and polychlorinated xanthenes that may be found at concentrations several orders of magnitude higher than the analytes of interest. Retention times of target analytes must be verified using reference standards. These values must correspond to the retention time windows established. While certain clean-up techniques are provided as part of this method, unique samples may require additional cleanup steps to achieve lower detection limits.

4.5. A high-resolution capillary column (60m DB-5) is used to resolve as many PCDD and PCDF isomers as possible. However, no single column is known to resolve all isomers. The DB-225 column is used for the quantitation of 2,3,7,8-TCDF.

5. SAFETY

5.1. Procedures shall be carried out in a manner that protects the health and safety of all associates.

5.2. Eye protection that satisfies ANSI Z87.1 (as per the Corporate Safety Manual), laboratory coat, and chemically resistant gloves must be worn while samples, standards, solvents, and reagents are being handled. Disposable gloves that have been contaminated will be removed and discarded; other gloves will be cleaned immediately.

5.2.1. N-Dex nitrile gloves provide varying degrees of intermittent splash protection against those chemicals listed. Refer to permeation/degradation charts for the actual data.

5.3. The health and safety hazards of many of the chemicals used in this procedure have not been fully defined. Additional health and safety information can be obtained from the Material Safety Data Sheets (MSDS) maintained in the laboratory.

5.3.1. Chemicals that have been classified as **carcinogens**, or **potential carcinogens**, under OSHA include: methylene chloride.

5.3.2. Chemicals known to be **flammable** are: toluene, hexane, acetone. Hexane is also a neurotoxic agent.

5.3.3. The following materials are known to be **corrosive**: H_2SO_4, NaOH, KOH.

5.3.4. 2,3,7,8-TCDD is identified as a carcinogen, teratogen, and mutagen. Other PCDDs and PCDFs containing chlorine atoms in positions 2,3,7,8 are known to have toxicities comparable to that of 2,3,7,8-TCDD.

5.4. Exposure to chemicals must be maintained **as low as reasonably achievable**, therefore, unless they are known to be non-hazardous, all samples must be opened, transferred and prepared in a fume hood. Solvent and waste containers will be kept closed unless transfers are being made.

5.5. The preparation of standards and reagents will be conducted in a fume hood with the sash closed as far as the operation will permit.

5.6. All work must be stopped in the event of a known or potential compromise to the health and safety of a STL Sacramento associate. The situation must be reported **immediately** to a laboratory supervisor.

5.7. The analyst should note that finely divided dry soils contaminated with PCDDs and PCDFs are particularly hazardous because of the potential for inhalation and ingestion. Such samples are to be processed in a confined environment, such as a hood or a glove box. If additional protection (i.e. respirators) are required, associates must notify the Environmental Health and Safety (EH&S) Coordinator. A hazard assessment must be conducted. Only the EH&S Coordinator is able to select and issue respiratory protection.

5.8. Safety practices described in Section 5.9 are adapted from EPA Method 613, Section 4 (July 1982 version).

5.9. Each laboratory must develop a strict safety program for the handling of 2,3,7,8-TCDD. The laboratory practices listed below are recommended.

5.9.1. Contamination of the laboratory will be minimized by conducting the manipulations in a fume hood.

5.9.2. The effluents of sample splitters for the gas chromatograph and roughing pumps on the HRGC/HRMS system should pass through either a column of activated charcoal or be bubbled through a trap containing oil or high-boiling alcohols.

6. EQUIPMENT AND SUPPLIES

6.1. High-Resolution Gas Chromatograph/High-Resolution Mass Spectrometer/Data System (HRGC/HRMS/DS).

6.1.1. The GC must be equipped for temperature programming. All required accessories must be available, such as syringes, gases, and capillary columns. The GC injection port must be designed for capillary columns. The use of splitless injection techniques is recommended. The use of a moving needle injection port is also acceptable. When using the method described in this protocol, a 2-µL injection volume is used consistently (i.e., the injection volumes for all extracts, blanks, calibration solutions and the performance check samples are 2 µL). 1 µL injections are allowed; however, laboratories are encouraged to remain consistent throughout the analyses by using the same injection volume at all times on a given HRGC/HRMS/DS.

6.1.2. Gas Chromatograph/Mass Spectrometer (GC/MS) Interface - The GC/MS interface components should withstand 350° C. The interface must be designed so that the separation of 2,3,7,8-TCDD from the other TCDD isomers achieved in the gas chromatographic column is not appreciably degraded. Cold spots or active surfaces (adsorption sites) in the GC/MS interface can cause peak tailing and peak broadening. It is recommended that the GC column be fitted directly into the mass spectrometer ion source without being exposed to the ionizing electron beam. Graphite ferrules should be avoided in the injection port because they may adsorb the PCDDs and PCDFs. Vespel® or equivalent ferrules are recommended.

6.1.3. Mass Spectrometer - The static resolving power of the instrument must be maintained at a minimum of 10,000 (10 percent valley). The mass spectrometer must be operated in a selected ion monitoring (SIM) mode with a total cycle time (including the voltage reset time) of one second or less.

6.1.4. Data System - A dedicated data system is employed to control the rapid multiple ion monitoring process and to acquire the data. Quantification data (peak areas or peak heights) and SIM traces (displays of intensities of each ion signal being monitored including the lock-mass ion as a function of time) must be acquired during the analyses and stored. Quantifications may be reported based upon computer-generated peak areas or upon measured peak heights (chart recording). The data system must be capable of acquiring data for a minimum of 10 ions in a single scan. It is also recommended to have a data system capable of switching to different sets of ions (descriptors) at specified times during an HRGC/HRMS acquisition. The data system should be able to provide hard copies of individual ion chromatograms for selected gas chromatographic time intervals. It should also be able to acquire mass-spectral peak profiles and provide hard copies of peak profiles to demonstrate the required resolving power. The data system should also permit the measurement of noise on the base line.

6.2. GC Column

6.2.1 In order to have an isomer-specific determination for 2,3,7,8-TCDD and to allow the detection of OCDD/OCDF within a reasonable time interval in one HRGC/HRMS analysis, the 60-m DB-5 fused-silica capillary column is recommended. At the beginning of each 12-hour period during which samples are analyzed and after tuning, acceptable compound separation on the GC column must be demonstrated through the analysis of a column performance check solution. Operating conditions known to produce acceptable results with the recommended column are shown in Table 7.

6.3. Miscellaneous Equipment and Materials

The following list of items does not necessarily constitute an exhaustive compendium of the equipment needed for this analytical method.

6.3.1. Nitrogen evaporation apparatus with variable flow rate.

6.3.2. Balances capable of accurately weighing to 0.01 g and 0.0001 g.

6.3.3. Centrifuge.

6.3.4. Water bath, equipped with concentric ring covers and capable of maintaining temperature control within ± 2°C.

6.3.5. Stainless steel or glass containers large enough to hold contents of one-pint sample containers.

6.3.6. Glove box.

6.3.7. Drying oven.

6.3.8. Stainless steel spoons and spatulas.

6.3.9. Laboratory hoods.

6.3.10. Pipettes, disposable, Pasteur, 150 mm long x 5 mm ID.

6.3.11. Pipettes, disposable, serological, 10 mL, for the preparation of the carbon column specified in Section 7.1.

6.3.12. Reacti-vial, 2 mL, silanized amber glass.

6.3.13. Stainless steel meat grinder with a 3- to 5-mm hole size inner plate.

6.3.14. Separatory funnels, 125 mL.

6.3.15. Kuderna-Danish concentrator, 500 mL, fitted with 10-mL concentrator tube and three-ball Snyder column.

6.3.16. Teflon® boiling chips (or equivalent), washed with DCM before use.

6.3.17. Chromatographic column, glass, 300 mm x 10.5 mm, fitted with Teflon® stopcock.

6.3.18. Adapters for concentrator tubes.

6.3.19. Glass fiber filters.

6.3.20. Dean-Stark trap, 5 or 10 mL, with T-joints, condenser and 125 mL flask.

6.3.21. Continuous liquid-liquid extractor.

6.3.22. All-glass Soxhlet apparatus, 500 mL flask.

6.3.23. Glass funnels, sized to hold 170 mL of liquid.

6.3.24. Desiccator.

6.3.25. Solvent reservoir (125 mL), Kontes; 12.35 cm diameter (special order item), compatible with gravity carbon column.

6.3.26. Rotary evaporator with a temperature controlled water bath.

6.3.27. High speed tissue homogenizer, equipped with an EN-8 probe or equivalent.

6.3.28. Glass wool, extracted with methylene chloride, dried and stored in a clean glass jar.

Note: Re-use of glassware should be minimized to avoid the risk of contamination. All glassware that is re-used must be scrupulously cleaned as soon as possible after use, applying the following procedure:

6.4. Proper cleaning of glassware is extremely important because glassware may not only contaminate the samples, but may also remove the analytes of interest by adsorption on the glassware surface.

6.4.1. Glassware should be rinsed with solvent and washed with a detergent solution as soon after use as is practical. Sonication of glassware containing a detergent solution for approximately 30 seconds may aid in cleaning. Glassware with removable parts, particularly separatory funnels with Teflon stopcocks, must be disassembled prior to detergent washing.

6.4.2. After detergent washing, glassware should be immediately rinsed with acetone, toluene, hexane, and then methylene chloride.

6.4.3. Do not kiln reusable glassware in an oven as a routine part of cleaning. Kilning may be warranted after particularly dirty samples are encountered, but should be minimized, as repeated kilning of glassware may cause the formation of active sites

on the glass surface that will irreversibly adsorb PCDDs/ PCDFs.

6.4.4. Immediately prior to use, Soxhlet extraction glassware should be pre-extracted with toluene for a minimum of 3 hours.

7. REAGENTS AND STANDARDS

7.1. Column Chromatography Reagents

7.1.1. Silica Gel - Kieselgel 60 or equivalent, activate for 1 hour at 184°C before use. Store at 130°C in covered flask.

7.1.2. Acid Alumina - ICN or equivalent, activated as necessary.

7.1.3. Basic Alumina - ICN or equivalent. No activation required.

7.1.4. Granular carbon/silica gel - Mix 3.6 g granular carbon and 16.4 g activated silica gel; (alternatively, prepare AX-21/silica gel (5%/95%); i.e., combine 5 g precleaned AX-21 with 95 g silica gel). Activate mix for >12 hours at 130°C before use. Store at 130°C in covered flask. The first LCS prepared with a new batch of column packing material is the quality control check of the packing materials. Refer to historical control limits before accepting the new batch of material.

7.1.5. 44% H_2SO_4 /silica gel - Mix 24 mL conc. H_2SO_4 and 56 g activated silica gel. Stir and shake until free flowing. Store at room temperature.

7.1.6. 33% NaOH/silica gel - Mix 34 mL 1N NaOH and 67 g activated silica gel. Stir and shake until free flowing. Store at room temperature.

7.2. Reagents

7.2.1. Sulfuric acid, concentrated, ACS grade, specific gravity 1.84.

7.2.2. Potassium hydroxide, ACS grade, 20 percent (w/v) in distilled water.

7.2.3. Distilled water demonstrated to be free of interferents

7.2.4. Potassium carbonate, anhydrous, analytical reagent.

7.2.5. Silica gel.

7.2.6. Solution for breaking emulsions: Add 1.0L of reagent grade NaOH solution to an empty 2.0L NaOH container. Then add 1.0L of DI H2O to the container and leave the container in secondary containment with the lid off. ***(The solution will begin to heat so let the solution stand until equilibrium is met and the solution is at room temperature).*** When this process is complete, the solution will then be ready for use in the samples.

7.3. Desiccating Agent

7.3.1. Sodium sulfate, granular, anhydrous.

7.4. Solvents

7.4.1. High-purity, distilled-in-glass or highest available purity: Methylene chloride, hexane, benzene, methanol, tetradecane, isooctane, toluene, cyclohexane, and acetone.

7.5. All calibration, internal standard, clean up recovery standards, and spiking solutions are stable for one year from preparation. After 1 year, solutions may be revalidated. The revalidated solution may be used for an additional year, or until there is evidence of compound degradation or concentration. The revalidation must be performed using an unexpired, not previously validated solution from a second lot or second vendor.

7.5.1. Sealed ampules may be used until the manufacturer's expiration date is exceeded. If no expiration date is provided, then the expiration date will be 1 year from the date the ampule is opened.

7.6. Calibration Solutions

7.6.1. High-Resolution Concentration Calibration Solutions (Table 5) - Five tetradecane solutions containing unlabeled (totaling 17) and carbon-labeled (totaling 16) PCDDs and PCDFs at known concentrations are used to calibrate the instrument. The concentration ranges are homolog dependent, with the lowest values associated with the tetra chlorinated dioxins and furans (0.5 pg/μL) and the highest for the octachlorinated congeners (2000 pg/μL).

7.6.2. Individual isomers that make up the high-resolution concentration calibration solutions are obtained from commercial sources and prepared in the laboratory.

These standards are traceable back to EPA-supplied standard solutions.

7.6.3. Store the calibration solutions in appropriate containers and at room temperature in the dark.

7.7. GC Column Performance Check Solution

7.7.1. This solution contains the first and last eluting isomers for each homologous series from tetra- through hepta-chlorinated congeners. The solution also contains a series of other TCDD isomers for the purpose of documenting the chromatographic resolution. The 13C-2,3,7,8-TCDD is also present. The laboratory is required to use tetradecane as the solvent and adjust the volume so that the final concentration does not exceed 100 pg/µL per congener. Table 8 summarizes the qualitative composition (minimum requirement) of this performance evaluation solution for the DB-5 column.

7.7.2. For the DB-225 column, the column performance check solution contains a series of TCDF isomers in addition to the 2,3,7,8-TCDF. The solution is injected and evaluated at the start of each analytical sequence on the DB-225 column to ensure that 2,3,7,8-TCDF is resolved from its closest eluting isomers with a baseline-to-valley ratio of ≤ 25%. Table 8 summarizes the qualitative composition (minimum requirement) of this performance evaluation solution on for the DB-225 column.

7.8. Field Surrogate Solution (air matrices)

7.8.1. This solution contains one 13C labeled (for Method TO-9/TO-9A) or five 13C labeled (for Method 0023) analogs at the nominal concentration indicated in Table . It is used to assess sample collection and recovery procedures.

7.9. Sample Fortification Solution (Internal Standard)

7.9.1. This isooctane (or toluene) solution contains the nine internal standards at the nominal concentrations that are listed in Table 2. The solution contains at least one carbon-labeled standard for each homologous series, and it is used to measure the concentrations of the native substances. (Note that 13C-OCDF is not present in the solution.)

7.10. Recovery Standard Solution

7.10.1. This tetradecane solution contains two recovery standards (13C-1,2,3,4-TCDD

and 13C-1,2,3,7,8,HxCDD). An appropriate volume of this solution will be spiked into each sample extract before the final concentration step and HRGC/HRMS analysis.

7.10 Preparation and QC of PUF material

7.10.1 The PUF material is purchased pre-cut.

7.10.2 The PUFs are rinsed by Soxhlet with methylene chloride for a minimum of 16 hours and air dried for a minimum of 2 hours in a contaminant-free area.

7.10.3 One PUF from the rinsed batch is randomly selected to be the QC sample for the batch.

7.10.4 The PUF is loaded into a pre-cleaned Soxhlet extractor charged with toulene.

7.10.5 The 1613/8290 daily internal standard solution is spiked into the PUF and it is extracted for a minimum of 16 hours.

7.10.6 The Soxhlet extract is recovered and processed according to section 11.4.

7.10.7 The batch of PUF is considered acceptable if no target analytes are detected at or above the laboratory or project specific reporting limit.

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1. The sample collection, shipping, handling, and chain-of-custody procedures are not described in this document. Sample collection personnel will, to the extent possible, homogenize samples in the field before filling the sample containers. This should minimize or eliminate the necessity for sample homogenization in the laboratory. The analyst should make a judgment, based on the appearance of the sample, regarding the necessity for additional mixing. If the sample is clearly non-homogeneous, the entire contents should be transferred to a glass or stainless steel pan for mixing with a stainless steel spoon or spatula before removal of a sample portion for analysis.

8.2. Grab and composite samples must be collected in glass containers.

8.3. Ambient air samples are collected on a Quartz Fiber Filter followed by a glass sleeve containing a polyurethane foam plug.

8.4. Samples from stationary sources are collected on glass or quartz fiber filters and XAD-2 Resin. (See SAC-ID-0009 for sample preparation procedures).

8.5. Conventional sampling practices must be followed. Do not rinse the bottle with sample before collection. Sampling equipment must be free of potential sources of contamination.

8.6. Grinding or Blending of Fish Samples.

8.6.1. If not otherwise specified by the client, the whole fish (frozen) should be blended or ground to provide a homogeneous sample. The use of a stainless steel meat grinder with a 3- to 5-mm hole size inner plate is recommended. In some circumstances, analysis of fillet or specific organs of fish may be requested by the client. If so requested by the client, the above whole fish requirement is superseded.

8.6.2. Hearing protection **must** be worn when grinding samples.

8.7. With the exception of the fish tissues, which must be stored at - 20°C, all samples should be stored at 4°C ± 2, extracted within 30 days and completely analyzed within 45 days of collection. The 30 day hold time is recommended. PCDDs and PCDFs have demonstrated stability for greater than one year.

8.8. All extracts must be stored capped, in the dark, at room temperature (approximately 21°C to 28°C).

8.9. Soil, Sediment or Paper Sludge (Pulp) Percent Moisture Determination.

The percent moisture of soil or sediment samples showing detectable levels (see note below) of at least one 2,3,7,8-substituted PCDD/PCDF congener is determined according to the following recommended procedure.

Generally, depending on sample availability, a 5-10 g sample, weighed to three significant figures, is used for % solids determination. The sample is then dried to constant weight at 110°C± 10 in an adequately ventilated oven. Weigh the dried solid to three significant figures. Calculate and report the percent moisture on the appropriate form. Do not use this solid portion of the sample for extraction, but instead dispose of it as hazardous waste.

$$\text{Percent Moisture} = \frac{\text{Weight of wet soil - Weight of dry soil}}{\text{Weight of wet soil}} \times 100$$

8.10. Fish Tissue Lipid Content Determination

The percent lipid of fish samples is determined as follows:

Concentrate the extract from Section 11.3.5 on a rotary evaporator until constant weight is attained. The percent lipid is calculated using the following expression:

$$\text{Percent lipid} = \frac{\text{Weight of residue from extraction (in g)}}{\text{Weight of fish tissue portion (in g)}} \times 100$$

9. QUALITY CONTROL

9.1. One method blank (MB) must be extracted with every process batch of similar matrix, not to exceed twenty (20) samples. The method blank is an aliquot of laboratory reagent water processed in the same manner and at the same time as the associated samples. Corrective actions must be documented on a Non-Conformance memo, then implemented when target analytes are detected in the method blank above the reporting limit or when surrogate recoveries are outside control limits. Re-extraction of the blank, other batch QC, and the affected samples are required when the method blank is deemed unacceptable. The method blank contains a PUF plug prepared from the same batch as the field samples whenever possible.

9.1.1. If the accompanying samples are aqueous, use distilled water as a matrix. Take the method blank through all steps detailed in the analytical procedure.

9.1.2. Use sodium sulfate as the method blank laboratory matrix when solids are extracted. Take the MB through all steps detailed in the analytical procedure.

9.1.3. The method blank must be spiked prior to extraction with the same amount of 13C-labeled internal standards as added to samples.

9.1.4. If method blank contamination is present, check solvents, reagents, fortification solutions, apparatus and glassware to locate and eliminate the source of contamination before any further samples are extracted and analyzed.

9.1.4.1. OCDD is a ubiquitous laboratory contaminant. A method blank and the associated samples are deemed acceptable if the OCDD concentration is <5x the specified reporting limit. Flag data

appropriately. The analyst is expected to investigate and eliminate potential sources of systematic contamination.

9.1.4.2. If a target analyte is detected in the blank but the associated samples are ND (not detected), then the data may be reported, unless otherwise directed by the client. Note the action in the narrative.

9.1.4.3. If a target analyte is detected in the blank, but the concentration of the contaminant in the samples >10x the blank concentration, then the data may be reported, unless otherwise directed by the client. Note the action in the narrative.

9.1.5. If new batches of reagents or solvents contain interfering contaminants, purify or discard them.

9.2. A Laboratory Control Sample (LCS) must be extracted with every process batch of similar matrix, not to exceed twenty (20) samples. The LCS is an aliquot of laboratory matrix (e.g. water, Ottawa sand, sodium sulfate, PUF, XAD, etc.) spiked with analytes of known identity and concentration. The LCS must be processed in the same manner and at the same time as the associated samples. Corrective actions must be documented on a Non-Conformance memo, then implemented when recoveries of any spiked analyte is outside control limits provided on the LIMS or by the client. Re-extraction of the blank, other batch QC and all associated samples are required if the LCS is deemed unacceptable. See QA Policy 003 for specific acceptance criteria. When associated with PUF samples, the LCS should contain a PUF plug prepared from the same batch as the field samples whenever possible.

9.2.1. A LCS is deemed acceptable if control analytes are above control limits and the associated samples are ND, unless otherwise specified by the client. Note any actions in the narrative.

9.3. A Matrix Spike/Matrix Spike Duplicate (MS/MSD or MS/SD) pair are extracted at the client's request only, or per NELAP requirements. An MS/MSD pair are aliquots of a selected field sample spiked with analytes of known identity and concentration. When requested by the client, the MS/MSD pair shall be processed in the same manner and at the same time as the associated samples. Corrective actions must be documented on a Non-Conformance memo, then implemented when recoveries of any spike analyte is outside control limits provided on the LIMS or by the client. Re-extraction of the blank, an LCS, the selected field sample, the MS/MSD may be required after evaluation and review. Matrix Spike/ Matrix Spike Duplicates are not generally applicable for air samples due to the

difficulty in collecting identical or representative samples. An LCS/LCSD may be extracted to show precision of the extraction and analysis process.

9.3.1. Matrix Spike (MS): A sample, which is spiked with a known amount of the matrix spike fortification solution prior to the extraction step. The recoveries of the matrix spike compounds are determined; they are used to estimate the effect of the sample matrix upon the analytical methodology.

9.3.2. Matrix Spike Duplicate (MSD): A second portion of the same sample as used in the matrix spike analysis and which is treated like the matrix spike sample.

9.3.3. Locate the sample for the MS and MSD analyses (the sample may be labeled "double volume").

9.3.4. Add an appropriate volume of the matrix spike fortification solution, adjusting the fortification level as specified in Table 1, under IS Spiking Levels.

9.3.5. Analyze the MS and MSD samples as described in Section 11.

9.3.6. The results obtained from the MS and MSD samples (percent recovery and concentrations of 2,3,7,8-substituted PCDDs/PCDFs) should agree within 20 percent relative difference. Report all results and flag outliers.

9.3.7. Internal standard recoveries are flagged if they are outside the recovery goals. Re-extraction of affected samples should be performed if signal-to-noise for any internal standard is less than 10:1.

9.4. Duplicates

9.4.1. Upon client request, duplicates may be processed. Locate the sample specified for duplicate analysis, and prepare and analyze a second 10-g soil or sediment sample portion or 1-L water sample, or an appropriate amount of the type of matrix under consideration. Duplicate samples are not generally applicable for air samples due to the difficulty in collecting identical or representative samples. A duplicate injection of a sample extract may be performed to display instrument precision.

9.4.1.1. The results of the laboratory duplicates (percent recovery and concentrations of 2,3,7,8-substituted PCDD/PCDF compounds)

should agree within 25 percent relative difference. Report all results and flag outliers.

9.4.2. Internal standard recoveries are flagged if they are outside the recovery goals. Re-extraction of affected samples should be performed if signal-to-noise for any internal standard is less than 10:1.

9.5. Field Blanks

9.5.1. Each batch of samples may contain a field blank sample of nominally uncontaminated soil, sediment or water that is to be processed for analysis.

9.5.1.1. Weigh a 10-g portion or use 1 L (for aqueous samples) of the specified field blank sample and add the appropriate amount of internal standard to yield 100 pg/µL in the final extract.

9.5.1.2. Extract by using the procedures described in Section 11.3. As applicable, add the appropriate amount of recovery standard to yield 100 pg/µL in the final extract. Analyze a 1-2 µL aliquot of the concentrated extract.

9.5.1.3. Calculate the concentration of 2,3,7,8-substituted PCDDs/PCDFs and the percent recovery of the internal standards.

9.6. Rinsate Samples

9.6.1. In addition to the field blank, a batch of samples may include a rinsate, which is a portion of the solvent (usually trichloroethylene) that was used to rinse sampling equipment. The rinsate is analyzed to assure that the samples were not contaminated by the sampling equipment.

9.6.2. The rinsate sample must be processed like a regular sample.

9.6.2.1. Take a 100-mL (± 0.5 mL) portion of the sampling equipment rinse solvent (rinsate sample), filter, if necessary, and add the appropriate amount of internal standard to yield 100 pg/µL in the final extract.

9.6.3. Using appropriate methods, concentrate to approximately 10 mL.

9.6.4. Just before analysis, add the appropriate amount of recovery standard to yield 100

pg/µL in the final extract. Reduce the volume to a final volume of 20 µL, as necessary. No column chromatography is required.

9.6.5. Analyze an aliquot following the same procedures used to analyze samples.

9.6.6. Report percent recovery of the internal standard and the presence of any PCDD/PCDF compounds in pg/mL of rinsate solvent.

9.7. Surrogate/Clean Up Recovery Standard

9.7.1. A surrogate compound may be spiked into all air media samples prior to collection. For all other matrices, a clean up recovery standard is spiked following extraction and just prior to cleanup, in order to monitor relative loss of internal standard during both extraction and cleanup.

9.8. Internal Standards

9.8.1. Internal standards must be spiked into all samples, QC samples, and included in all calibrations.

9.8.2. For each sample and QC aliquot, calculate the percent recovery. The percent recovery should be between 40 percent and 135 percent for tetra-hexa 2,3,7,8-substituted internal standards, and 25 – 135 percent for hepta and octa 2,3,7,8-substituted internal standards.

9.8.3. A low or high percent recovery for a blank does not require discarding the analytical data but it may indicate a potential problem with future analytical data. Internal standard recoveries are flagged if they are outside the recovery goals. Re-extraction of affected samples should be performed if signal-to-noise for any internal standard is less than 10:1.

9.9. Recommended Corrective Actions and Troubleshooting Steps

- Verify satisfactory instrument performance.
- If possible, verify that no error was made while weighing the sample portions.
- Review the analytical procedures with the performing laboratory personnel.

9.10. Identification Criteria

9.10.1. If either one of the identification criteria appearing in Sections 11.5.4.1 is not met for an homologous series, it is reported that the sample does not contain unlabeled 2,3,7,8-substituted PCDD/PCDF isomers for that homologous series at the calculated detection limit.

9.10.2. If the criteria specified in sections 11.5.4.1 and 11.5.4.2 are not met (i.e. retention time and ion abundance ratios), that sample is presumed to contain interfering contaminants. This must be noted on the analytical report form. The sample may be rerun or the extract reanalyzed as directed by the client.

10. CALIBRATION AND STANDARDIZATION

Calibration and Standardization requires a check of mass resolution (tuning), a check of chromatographic resolution, a verification of switching times (i.e. descriptors), and a calibration curve verification.

10.1. Tuning (Mass Resolution Check)

10.1.1. The mass spectrometer must be operated in the electron ionization mode. A static resolving power of at least 10,000 (10 percent valley definition) must be demonstrated at appropriate masses before any analysis is performed. Corrective actions must be implemented whenever the resolving power does not meet the requirement.

10.1.2. Chromatography time for PCDDs and PCDFs exceeds the long-term mass stability of the mass spectrometer. Because the instrument is operated in the high-resolution mode, mass drifts of a few ppm (e.g., 5 ppm in mass) can have serious adverse effects on instrument performance. Therefore, a mass-drift correction is mandatory. To that effect, it is recommended to select a lock-mass ion from the reference compound (PFK is recommended) used for tuning the mass spectrometer. The selection of the lock-mass ion is dependent on the masses of the ions monitored within each descriptor. Table 6 offers some suggestions for the lock-mass ions. However, an acceptable lock-mass ion at any mass between the lightest and heaviest ion in each descriptor can be used to monitor and correct mass drifts. The level of the reference compound (PFK) metered into the ion chamber during HRGC/HRMS analyses should be adjusted so that the amplitude of the most intense selected lock-mass ion signal (regardless of the descriptor number) does not exceed 10 percent of the full-scale deflection for a given set of detector parameters. Under those conditions, sensitivity changes that might occur

during the analysis can be more effectively monitored.

NOTE: Excessive PFK (or any other reference substance) may cause noise problems and contamination of the ion source resulting in downtime for source cleaning.

10.1.3. By using a PFK molecular leak, tune the instrument to meet minimum required resolving power of 10,000 (10 percent valley) at m/z 304.9824 (PFK) or any other reference signal close to m/z 303.9016 (from TCDF). Verify that the exact mass of m/z 380.9760 (PFK) is within 5 ppm of the required value. Note that the selection of the low- and high-mass ions must be such that they provide the largest voltage jump performed in any of the five mass descriptors (Table 6).

10.1.4. Documentation of the instrument resolving power must then be accomplished by recording the peak profile of the high-mass reference signal (m/z 380.9760). The minimum resolving power of 10,000 must be demonstrated on the high-mass ion while it is transmitted at a lower accelerating voltage than the low-mass reference ion, which is transmitted at full sensitivity. The format of the peak profile representation (Figure 6) must allow manual determination of the resolution, i.e., the horizontal axis must be a calibrated mass scale (amu or ppm per division). The result of the peak width measurement (performed at 5 percent of the maximum, which corresponds to the 10-percent valley definition) must appear on the hard copy and cannot exceed 100 ppm at m/z 380.9760 (or 0.038 amu at that particular mass).

10.2. Performance Checks

10.2.1. At the beginning of each 12-hour period during which samples are to be analyzed, aliquots of the 1) GC column performance check solution and 2) high-resolution concentration calibration solution No. 3 (HRCC-3) shall be analyzed to demonstrate adequate GC resolution and sensitivity, response factor reproducibility, and mass range calibration, and to establish the PCDD/PCDF retention time windows. A mass resolution check shall also be performed to demonstrate adequate mass resolution using an appropriate reference compound (PFK is recommended). If the required criteria not met, remedial action must be taken before any samples are analyzed. The mass resolution check will be taken at the beginning and completion of an analytical sequence. An analytical sequence may contain one or more 12 hour period.

10.2.1.1. Method blanks or solvent blanks are used to demonstrate that the analytical system is free of contamination after the analysis of calibration standards or high level samples. The blank must demonstrate that the system has returned to appropriate background levels prior to continued analysis.

10.2.2. At a minimum, the ions listed in Table 6 for each of the five SIM descriptors must be monitored. Note that the PeCDF masses (M−2 & M+4) are also monitored in the first descriptor. This is because the first PeCDF isomer elutes closely to the final tetra isomer. The selection (Table 6) of the molecular ions M and M+2 for 13C-HxCDF and 13C-HpCDF rather than M+2 and M+4 (for consistency) is to eliminate, even under high-resolution mass spectrometric conditions, interferences occurring in these two ion channels for samples containing high levels of native HxCDDs and HpCDDs. It is important to maintain the same set of ions for both calibration and sample extract analyses. The recommended mass spectrometer tuning conditions are based on the groups of monitored ions shown in Table 6.

10.2.2.1. The GC column performance check mixture, high-resolution concentration calibration solutions, and the sample fortification solutions may be obtained from the EMSL-CIN. However, if not available from the EMSL-CIN, standards can be obtained from other sources, and solutions can be prepared in the laboratory. Concentrations of all solutions containing 2,3,7,8-substituted native PCDDs/PCDFs, must be verified by comparison with second-source standard solutions.

10.3. Initial Calibration

Initial calibration is required before any samples are analyzed for PCDDs and PCDFs. Initial calibration is also required if any routine calibration (Section 10.5) does not meet the required criteria listed in Section 10.6.

10.3.1. Five high-resolution concentration calibration solutions, listed in Table 5, must be used for the initial calibration.

10.3.2. Tune the instrument with PFK.

10.3.3. Inject 1 or 2 µL of the GC column performance check solution and acquire SIM mass spectral data as described earlier in Section 6.1.3. The total cycle time must be ≤ 1 second. The laboratory must not analyze samples until it is demonstrated and documented that the criterion listed in Section 13.1 is met.

10.3.3.1. Select the injection volume based upon the expected target analyte concentration, or expected matrix interferences.

10.3.3.2. The same injection volume must be used for all samples, QC, and standards.

10.3.4. By using the same GC and mass spectrometer conditions that produced acceptable results with the column performance check solution, analyze a 1 or 2-µL portion of each of the five concentration calibration solutions once with the following mass spectrometer operating parameter.

10.3.4.1. The total cycle time for data acquisition must be < 1 second. The total cycle time includes the sum of all dwell times and voltage reset times.

10.3.4.2. Acquire SIM data for all the ions listed in the five descriptors of Table 6.

10.3.4.3. The ratio of integrated ion current for the ions appearing in Table 9 (homologous series quantification ions) must be within the indicated control limits (set for each homologous series).

10.3.4.4. The ratio of integrated ion current for the ions belonging to the 13C labeled internal and recovery standards must be within the control limits stipulated in Table 9.

NOTE: Section 10.4.3 requires that ion ratios be within the specified control limits simultaneously in one run. It is the laboratory's responsibility to take corrective action if the ion abundance ratios are outside the limits.

10.3.5. For each SICP and for each GC signal corresponding to the elution of a target analyte and of its labeled standards, the signal-to-noise ratio (S/N) must be better than or equal to 10. This measurement is suggested for any GC peak that has an apparent S/N of less than 5:1. The result of the calculation must appear on the SICP above the GC peak in question.

10.3.5.1. Referring to Table 10, calculate the 17 relative response factors (RRF) for unlabeled target analytes [RRF(n); n=1 to 17] relative to their appropriate internal standards (Table 5) and the nine RRFs for

the labeled 13C internal standards [RRF(m); m=18 to 26] relative to the two recovery standards according to the following formulae:

$$RRF(n) = \frac{A_x \times Q_{is}}{Q_x \times A_{is}}$$

$$RRF(m) = \frac{A_{is} \times Q_{rs}}{Q_{is} \times A_{rs}}$$

Where:

A_x = sum of the integrated ion abundances of the quantitation ions (Tables 6 and 10) for unlabeled PCDDs/PCDFs,

A_{is} = sum of the integrated ion abundances of the quantitation ions (Tables 6 and 10) for the labeled internal standards,

A_{rs} = sum of the integrated ion abundances of the quantitation ions (Tables 6 and 10) for the labeled recovery standards,

Q_{is} = quantity of the internal standard injected (pg),

Q_{rs} = quantity of the recovery standard injected (pg), and

Q_x = quantity of the unlabeled PCDD/PCDF analyte injected (pg).

The RRF(n) and RRF(m) are dimensionless quantities; the units used to express Qis, Qrs, and Qx must be the same.

10.3.5.2. Calculate the RRF(n)s and their respective percent relative standard deviations (%RSD) for the five calibration solutions:

$$\overline{RRF}(n) = (1/5) \sum_{j=1}^{5} RRF_j(n)$$

Where n represents a particular PCDD/PCDF (2,3,7,8-substituted) congener (n = 1 to 17; Table 10), and j is the injection number (or calibration solution number; j = 1 to 5).

10.3.5.3. The relative response factors to be used for the determination of the concentration of total isomers in a homologous series (Table 10) are calculated as follows:

10.3.5.3.1. For congeners that belong to a homologous series containing only one isomer (e.g., OCDD and OCDF) or only one 2,3,7,8-substituted isomer (Table 4; TCDD, PeCDD, HpCDD, and TCDF), the mean RRF used will be the same as the mean RRF determined in Section 10.3.5.2.

NOTE: The calibration solutions do not contain 13C-OCDF as an internal standard. This is because a minimum resolving power of 12,000 is required to resolve the [M+6]+ ion of 13C-OCDF from the [M+2]+ ion of OCDD (and [M+4]+ from 13C-OCDF with [M]+ of OCDD). Therefore, the RRF for OCDF is calculated relative to 13C-OCDD.

10.3.5.3.2. For congeners that belong to a homologous series containing more than one 2,3,7,8-substituted isomer (Table 4), the mean RRF used for those homologous series will be the mean of the RRFs calculated for all individual 2,3,7,8-substituted congeners using the equation below:

$$\overline{RRF}(k) = (1/t)\sum_{n=1}^{t} RRF_n$$

Where:

k = 27 to 30 (Table 10), with 27 = PeCDF;
28 = HxCDF; 29 = HxCDD; and 30 = HpCDF,
t = total number of 2,3,7,8-substituted isomers present in the calibration solutions (Table 5) for each homologous series (e.g., two for PeCDF, four for HxCDF, three for HxCDD, two for HpCDF).

NOTE: Presumably, the HRGC/HRMS response factors of different isomers within a homologous series are different. However, this analytical protocol will make the assumption that the HRGC/HRMS responses of all isomers in a homologous series that do not have the 2,3,7,8-substitution patterns are the same as the responses of one or more of the 2,3,7,8-substituted isomer(s) in that homologous series.

10.3.5.4. Relative response factors [RRF(m)] to be used for the determination of the percent recoveries for the nine internal standards are calculated as follows:

$$RRF(m) = \frac{A_{is}^{m} \times Q_{rs}}{Q_{is}^{m} \times A_{rs}}$$

$$\overline{RRF}(m) = (1/5)\sum_{j=1}^{5} RRF_j(m)$$

Where:

m = 18 to 26 (congener type)
j = 1 to 5 (injection number),
A_{is}^{m} = sum of the integrated ion abundances of the quantitation ions (Tables 6 and 10) for a given internal standard (m = 18 to 26),
A_{rs} = sum of the integrated ion abundances of the quantitation ions (Tables 6 and 10) for a given internal standard (m = 18 to 26),
Q_{rs} & Q_{is}^{m} = quantities of, respectively, the recovery standard (rs) and a particular internal standard (m) injected (pg),
RRF(m) = relative response factor of a particular internal standard (m) relative to an appropriate recovery standard, as determined from one injection, and

$\overline{RRF(m)}$ = calculated mean relative response factor of a particular internal standard, as determined from the five initial calibration injections (j).

10.4. Criteria for acceptable calibration

The criteria listed below for acceptable calibration must be met before sample analysis is performed.

10.4.1. The percent relative standard deviations for the mean response factors [RRF(n) and RRF(m)] from the 17 unlabeled standards must be ≤ 20 percent, and those for the nine labeled reference compounds must be ≤ 30 percent.

10.4.2. The signal/noise ratio (S/N) for the GC signals present in every SICP (including the ones for the labeled standards) must be ≥ 10.

10.4.3. The isotopic ratios (Table 9) must be within the specified control limits.

NOTE: If the criterion for acceptable calibration listed in Section 10.4.1 is met, the analyte-specific RRF can then be considered independent of the analyte quantity for the calibration concentration range. The mean RRFs will be used for all calculations until the routine calibration criteria (Section 10.6) are no longer met. At such time, new mean RRFs will be calculated from a new set of injections of the calibration solutions.

10.5. Routine Calibration (continuing calibration check)

Routine calibrations must be performed at the beginning of (following a successful tune and GC column performance check) and after a 12 hour period. The routine calibration initiates the 12 hour clock during which samples may be subsequently analyzed. The last sample in the sequence must be injected within 12 hours of the routine calibration, followed by the analysis of a closing calibration check. An acceptable closing calibration check standard may be used to initiate the next 12 hour analysis sequence when consecutive acquisition sequences occur. The ending mass resolution check shall be performed after the closing calibration check of an analysis acquisition sequence or after the final bracketing standard when consecutive 12 hour acquisition sequences are run.

10.5.1. Inject 1 or 2 µL of the concentration calibration solution HRCC-3 containing 10 pg/µL of tetrachlorinated congeners, 50 pg/µL of penta-, hexa-, and heptachlorinated congeners, 100 pg/µL of octachlorinated congeners, and the respective internal and recovery standards (Table 5). By using the same HRGC/HRMS conditions as used in Sections 6.1.3 through 6.2, determine and document an acceptable calibration as provided in Section 10.6.

10.6. Criteria for Acceptable Routine Calibration

The following criteria must be met before further analysis is performed. If these criteria are not met, corrective action must be taken, including recalibration if needed.

10.6.1. The measured RRFs [RRF(n) for the unlabeled standards] obtained during the opening continuing calibration must be ± 20 percent of the mean values established during the initial calibration (Section 10.3.5.)

10.6.1.1. The bracketing continuing calibration must be ± 25% of the average RRF calculated from the initial calibration.

10.6.2. The measured RRFs [RRF(m) for the labeled standards] obtained during the opening continuing calibration must be ± 30 percent of the mean values established during the initial calibration (Section 10.1.5).

10.6.2.1. The bracketing continuing calibration must be + 35% of the average RRF calculated from the initial calibration.

10.6.3. The ion-abundance ratios (Table 9) must be within the allowed control limits.

10.6.4. If either one of the above criteria (Sections 10.6.1 and 10.6.2) is not satisfied, the entire initial calibration process (Section 10.3) must be repeated. If either criteria in Sections 10.6.1.1 or 10.6.2.1 are not met, additional samples may not be analyzed. Sample data collected must be evaluated for usability. Narrate any reported data from the analytical sequence. If the ion-abundance ratio criterion is not satisfied, refer to the note in Section 10.3.4.4 for resolution.

11. PROCEDURE

11.1. One time procedural variations are allowed only if deemed necessary in the professional judgment of supervision to accommodate variation in sample matrix, radioactivity, chemistry, sample size, or other parameters. Any variation in procedure shall be completely documented using a Nonconformance Memo and is approved by a Technical Specialist and QA Manager. If contractually required, the client shall be notified. The Nonconformance Memo shall be filed in the project file.

11.2. Any unauthorized deviations from this procedure must also be documented as a nonconformance, with a cause and corrective action described.

11.3. Extraction

11.3.1. Internal Standard Addition. A 2000 pg aliquot of the internal standard mixture is added to all samples, regardless of sample size. As an example, for 13C-2,3,7,8-TCDD, a 10-g soil sample requires the addition of 2000 pg of 13C-2,3,7,8-TCDD to give the requisite fortification level.

11.3.2. Sludges. Paper Pulp Sludges are generally air-dried and ground. Because of the drying procedure, a Dean-Stark water separator is optional for extraction.

Extraction is generally done by Soxhlet with 200-300 mL of toluene.

11.3.2.1. Non-Paper Pulp Sludges are extracted with 200-300 mL of toluene.

11.3.2.2. Extract the sample for a minimum of 16 hours. Cool the sample, filter the toluene extract, if needed, through a glass-fiber filter or equivalent into a round-bottom flask. Rinse the filter with 10 mL toluene, and combine the extract and rinsate. Concentrate the combined solutions to near dryness on a rotary evaporator at 50°C. Use of an inert gas to concentrate the extract is also permitted. Proceed with Section 11.6 as necessary.

11.3.3. Still-Bottom/Fuel Oil. All organic liquids, fuel oils, and solids that will dissolve in a solvent and are treated as a solvent dilution will be dissolved in 1-2 mL of an appropriate solvent; then diluted with 5-10 mL of Hexane if no acid wash is required. Spike with appropriate Internal Standards and proceed with Section 11.6 as necessary.

11.3.4. Fly Ash. Extract fly ash samples by jar shake with hydrochloric acid before Soxhlet extraction. Weigh 2-10g of sample aliquot into a clean glass jar. Add 1.0mL of the internal standard mixture with 2 mL of acetone. Add 150 mL of 1N hydrochloric acid and shake for 4 hours. If the sample reacts violently with acid, then allow the sample to equilibrate for 4 hours with no shaking. The contents of the jar are then filtered. The solids are Soxhlet extracted for 16 hours. The aqueous filtrate is extracted with 100 mL of toluene and twice with 100 mL of hexane. Concentrate the combined toluene solutions to near dryness on a rotary evaporator at 50°C. Proceed with Section 11.6 as necessary.

Note: As an option, a Soxhlet/Dean Stark extractor system may be used, with toluene as the solvent. No sodium sulfate is added when using this option.

11.3.5. Solids/Tissues. Add anhydrous sodium sulfate to the soil sample portion in a ratio of 2 to 1 (e.g. 20 g sodium sulfate to 10 g of sample) and mix thoroughly with a stainless steel spatula. After breaking up any lumps, place the soil/sodium sulfate mixture in the Soxhlet apparatus on top of a glass-wool plug (the use of an extraction thimble is optional). Add 200 to 250 mL toluene to the Soxhlet apparatus and reflux for 16 hours. The solvent must cycle completely through the system at least five times per hour. Proceed with Section 11.6.

11.3.6. Preparation of stationary source samples. See SOP SAC-ID-0009.

11.4. Preparation of Crawfish Samples

11.4.1. Prior to dissecting the crawfish, rinse the animal with reagent water.

11.4.2. Isolate the crawfish abdomen by manually pulling the abdomen away from the cephalothorax.

11.4.3. Using forceps, hold the abdomen down and make a ventral incision of the cuticle along the full length of the abdomen midline using either dissection scissors or a scalpel blade. If using a scalpel blade, cut from the hand toward the tail.

11.4.4. Hold open the abdomen midline incision and remove the abdominal flexor muscle (tail meat) with forceps and place in a weighing boat.

11.4.5. If any of the anterior portion of the abdominal flexor muscle remained in the cephalothorax, remove it with forceps and combine with the muscle portion from 11.4.4. The combined portion should yield 3-6 grams of meat.

11.4.6. With a spatula, remove a portion of the hepatopancreous from the cephalothorax. An attempt should be made to recover as much of the hepatopancreous as possible. This should yield a few hundred milligrams of tissue. (Test yielded approximately 0.5-0.8 g wet weight of hepatopancreous from medium sized crawfish.)

11.4.7. Combine the hepatopancreous with the abdominal flexor muscle tissue. (Tests yielded approximately 5 g total wet weight of combined tissue per crawfish specimen using 12-18 g animals.)

11.4.8. Repeat steps 11.4.1 through 11.4.6 with additional crawfish specimens and composite the tissue until sufficient tissue mass is acquired for sample preparation and analysis as specified in the appropriate laboratory SOPs. (The composited tissue may be required for more than just the Dioxin analysis described in this SOP).

11.4.9. Record the composited tissue mass collected.

Note: The laboratory may elect to perform a combination of Dioxin/Furans and PCBs, in which case a 20 gram aliquot of the sample will be extracted and split prior to extract cleanup.

11.4.10. Homogenize the composited tissue and proceed with removal of a sample aliquot for extraction and analysis as directed in Section 11.4.11.

11.4.11. Weigh approximately a 10 gram aliquot into an extraction thimble and record the sample weight on the extraction sheet.

11.4.12. Place the extraction thimble containing the sample in a pre-cleaned Soxhlet extractor charged with 200-220 mL of fresh toluene and boiling chips.

11.4.13. Proceed with the extraction according to section 11.3.5.

Note: If a % lipid determination is required a tared round bottom flask is used. There is no solvent keeper added prior to extract concentration by rotary evaporation and the sample is reduced to dryness and reweighed according to section 8.11. The sample is then redissolved in hexane and process to section 11.6.2.

11.4.14. Aqueous Samples. Mark the water meniscus on the side of the 1-L sample bottle for later determination of the exact sample volume. Pour the entire sample (approximately 1-L) into a 2-L separatory funnel.

11.4.14.1. Add 100 mL methylene chloride to the sample bottle, seal, and shake for 30 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel. The internal standard mixture is first mixed in 2 mL of acetone then it is added to the sample in the separatory funnel. Each aliquot of spike mixture is added similarly. Extract the sample by shaking the funnel for two minutes with periodic venting. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. Extraction is repeated two additional times with methylene chloride.

11.4.14.2. Determine the original sample volume by filling the sample bottle to the mark with water and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11.4.14.3. Dry extract with sodium sulfate: Place glass wool in a precleaned filter funnel. Rinse glass wool with DCM and load funnel with DCM-

rinsed Na2SO4. Pour extract through Na2SO4 to remove water. Rinse Na2SO4 with fresh DCM and collect in round bottom flask.

11.4.14.4. Transfer the extract to a 500-mL round-bottom, add approximately 100 µL of tetradecane and concentrate on a rotary evaporator or TurboVap.

11.4.15. A continuous liquid-liquid extractor may be used in place of a separatory funnel when experience with a sample from a given source indicates that a serious emulsion problem will result or an emulsion is encountered when using a separatory funnel.

11.4.15.1. Add 60 mL methylene chloride to the sample bottle, seal, and shake for 30 seconds to rinse the inner surface.

11.4.15.2. Transfer the solvent to the extractor.

11.4.15.3. Repeat the sample bottle rinse with an additional 50- to 100-mL portion of methylene chloride and add the rinsate to the extractor.

11.4.15.4. Add 200 to 500 mL methylene chloride to the distilling flask, add sufficient reagent water to ensure proper operation, and extract for 24 hours.

11.4.15.5. Allow to cool, then detach the distilling flask.

11.4.16. Filter/PUF Samples

11.4.16.1. Place the glass sleeve containing the PUF and the Quartz Fiber Filter into the pre-cleaned Soxhlet extractor charged with toluene.

11.4.16.2. Add 2 mL (4000 pg) of 1613/8290 Internal Standard solution to all samples and QC.

11.4.16.3. Add 50 mL of 1613/8290 Native Spike to the LCS.

11.4.16.4. Extract the samples and QC for a minimum of 16 hours.

11.4.16.5. Concentrate the extract from the round bottom flask with hexane and adjust the volume.

11.4.16.6. Transfer the extract from the round bottom flask with hexane and adjust the volume.

11.4.16.7. Split the extract 50:50 for analysis and archive.

11.4.16.8. Proceed to Section 11.6.

11.5. There are several useful methods to decrease or eliminate emulsion in aqueous samples when extracting with DCM. These methods may include stirring with a pipet to manually breakup the emulsions or to transfer the sample into centrifuge tubes and centrifuge at approximately 3000 RPM. The most useful method is to use a 1:1 NaOH/H2O solution to change the pH enough to disrupt the emulsion phase, which works 90% of the time. See section 7.2 for reagent preparation.

11.5.1. Breaking Emulsions:

11.5.1.1. Check the pH of the sample to verify that the pH is between 3 and 7. If the pH is greater than 7, consult the supervisor and client for instructions.

11.5.1.2. Pour approximately 100 mL of the 1:1 NaOH:H_2O into a 1.0L AGB.

11.5.1.3. Drain the sample with the emulsion from the 2.0L separatory funnel into the 1L AGB and let it stand.

11.5.1.4. Empty the aqueous waste into the LLE waste drum.

11.5.1.5. Pour the solution with DCM back into the same 2.0L separatory funnel and drain the DCM phase through Na2SO4 into a 500mL roundbottom flask.

11.5.1.6. Empty the aqueous waste into the LLE waste drum.

11.5.1.7. Proceed with section 11.6.

11.6. Optional Extract Clean-Ups

11.6.1. Acid/Base Partitioning

11.6.1.1. Partition the extract in 50-125 mL of hexane against 40 mL concentrated H2SO4 in a separatory funnel. Shake for two minutes. Remove and discard the sulfuric acid layer (bottom). Repeat the acid washing until no color is visible in the acid layer (perform a maximum of four acid washings).

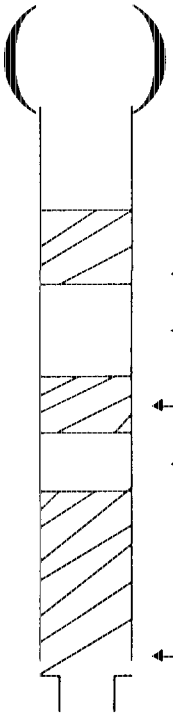
11.6.1.2. Partition the extract against 50 mL distilled H_2O. Shake for two minutes. Remove and discard the aqueous layer (bottom). If further cleanup is required, proceed to section 11.6.1.3

11.6.1.3. Partition the extract using 50 mL of 10 N NaOH. Shake for two minutes. Remove and discard the aqueous layer (bottom). Repeat the base washing until no color is visible in the bottom layer (perform a maximum of four base washings). Strong base is known to degrade certain PCDDs/PCDFs, so contact time must be minimized. The NaOH partition is applied only as samples warrant it at the discretion of the analyst.

11.6.1.4. Partition the extract against 50 mL of distilled H_2O. Shake for two minutes. Remove and discard the aqueous layer (bottom). Dry the extract by pouring it through a funnel containing anhydrous sodium sulfate and collect it in a round-bottom flask. Rinse the sodium sulfate with two 15-mL portions of hexane, add the rinsates to the flask, and concentrate the hexane solution to near dryness on a rotary evaporator (35°C water bath), making sure all traces of toluene (when applicable) are removed. (Use of blow-down with an inert gas to concentrate the extract is also permitted.) The DI H_20 partition is applied only as samples warrant it at the discretion of the analyst.

11.6.2. Silica Column Clean-Up "Exhibit A"

EXHIBIT A
IFB Column Clean-up



Use 20 mm column for top column

Use 16 mm column for bottom column*

	# of 15 mL capful
1 cm Na_2SO_4	1
2g activated Silica Gel	2
6-8g 44% H_2SO_4/Silica gel	4
2g activated Silica gel	2
glass wool	

*Upper and lower columns are piggy backed

11.6.2.1. Pre-rinse both columns with hexane - 20 mL Top and 20 mL Bottom.

11.6.2.2. Put one column above the other.

11.6.2.3. Add extract to the top column. Rinse extract vessel 2 times with 1 mL each of hexane and add to column.

11.6.2.4. Elute 60 mL hexane directly onto acid silica column (upper piggy backed columns).

11.6.2.5. Discard upper column.

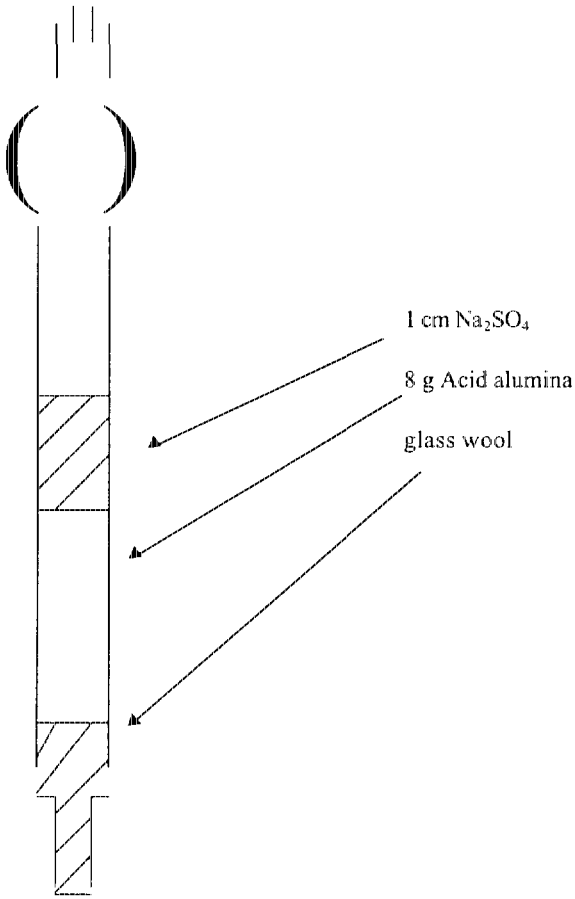
11.6.2.6. Elute lower column with 10 mL of 20% methylene chloride/hexane. Discard in proper waste stream.

11.6.2.7. Elute lower column with 30 mL of 65% methylene chloride/hexane. Save and collect in culture tube.

11.6.2.8. Proceed with additional cleanups as necessary.

11.6.3. Acid Alumina Column Clean-Up "Exhibit B"

Exhibit B
Acid Alumina Column Clean-up



11.6.3.1. Alumina activity may vary with the matrix or environmental conditions. Monitor internal standard and cleanup recovery standard recoveries in extract analysis. Low recoveries of cleanup recovery standard (CRS) may indicate loss of alumina activity. Assess stability of alumina activity and apply corrective action as appropriate (reactivate and reprofile).

11.6.3.2. Profile each vendor lot of activated alumina as corrective actions to low internal standard and CRS. recoveries dictate. If necessary, proceed as follows:

11.6.3.2.1. Set up and label 3 acid alumina columns.

11.6.3.2.2. Pre-rinse with 20 mL hexane.

11.6.3.2.3. Add 2 mL hexane spiked with internal standards and natives (spike amounts equivalent to those for LCS) with 2X2 mL hexane rinse of fractions.

11.6.3.2.4. Elute each column with 20 mL hexane. Collect and label these fractions.

11.6.3.2.5. Elute each column with 5 x10 mL methylene chloride/hexane at the appropriate v/v percent. Collect and label these fractions separately.

11.6.3.2.6. Elute each column with 10 mL of 100% methylene chloride. Collect and label these fractions. Reduce all fractions to final volume and add recovery standard.

11.6.3.3. Review data and select and elution scheme. Group the fraction from each solvent system as follows:

- Pre-analyte fraction - consists of all eluent prior to elution of first target analytes..
- Analyte fraction - consists of all which contain detectable levels of target analytes.
- Post-analyte fraction - consists of all eluents after elution of the last target analyte.

11.6.3.4. Select the solvent system which best meets the following two conditions:

- Pre-analyte fraction consists of 20 mL hexane and no more than 20 mL mixed solvent.
- Analyte fraction consists of no more than 20 mL of mixed solvent and contains greater than 90% of all target analytes and greater than 80% of all internal standards.

11.6.3.5. After selection of the appropriate solvent system and fractionation pattern, perform triplicate acid alumina cleanups on spiked hexane to ensure reproducibility of the fractionation pattern. Document each elution scheme and append to this SOP.

11.6.3.6. Each subsequent batch of acid alumina used in the lab (from the same vendor lot) must be checked for stable activity.

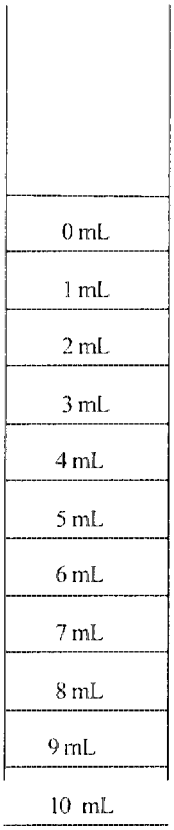
11.6.4. Carbon Column Clean-up "Exhibit C"

11.6.4.1. Prepare an activated Carbon & Silica Gel column as described in EXHIBIT C, below.

Exhibit C

Carbon Column Clean-up
Special D2

"A"



"B"

- Cut off both ends of a 10 mL pipette, or use pre-cut column.

- Push a glasswool plug down to the mark.

- Add 1g of 5% activated carbon/silica and top with another glasswool plug.

- Pre-elute with 5 mL 1:1 MeCl2:cyclohexane. Direction "A"
- Turn over and pre-elute with 5 mL 1:1 MeCl2:cyclohexane in direction "B".

- Discard pre-eluates.

- Dilute extract to 1 mL with hexane and transfer to the column.

- Rinse sample vial onto the column with 2 X 2 mL 1:1 MeCl2:cyclohexane.

- Elute with 6mL 1:1 MeCl2:cyclohexane
5mL 75:20:5 MeCl2:MeOH:Benzene

- Discard Evaluates

-After flipping column back to the "A" direction, the column is eluted with 30 mL of toluene and collected

- N2 or roto-vap to NEAR dryness and proceed to 11.8.

11.7. Sample Dilution Procedure

11.7.1. Simple dilutions: Dilutions from 2X to 50X can be achieved without respiking the final extract. The calculation to determine the final extract concentration is as follows:

(Concentration of the original extract) x (amount of aliquot taken) x (volume of diluted extract) = final concentration of dilution.

Ex: 50X dilution of original 10 g/20 uL sample
(10 g/20 uL) x (2 ul aliquot + 98 uL keeper) = 1 g/100 uL FV

Record the final sample concentration on the extract label.

11.7.2. Complex dilution requiring respiking of IS and RS: Dilutions greater than 50x must be done by diluting and respiking the extract with IS and RS. This procedure may require serial dilution to be performed. If this procedure is done, then the sample size must be adjusted to reflect the aliquot taken.

Ex. 100X dilution (original sample with 10 g/20 uL final volume)

Take a 2 uL aliquot (1/10 of original sample) and add 18 uL of solvent keeper. Take a 2 uL aliquot of the dilution (1/100 of the original sample), respike with 1 mL IS and 20 uL RS, reduced to 20 uL FV.

Record the final sample concentration of the extract label.

11.8. Analytical Procedures

11.8.1. With a stream of dry, purified nitrogen, reduce the extract volume to 10 µL. Add 20 µL of the recovery standard solution (Table 2). With a stream of dry, purified nitrogen, reduce the extract to volume to 20 µL.

11.8.1.1. Transfer the extract to an autoinjection vial and store in the dark at room temperature.

11.8.2. Inject a 1 or 2 µL aliquot of the extract into the GC, operated under the conditions previously used (Section 6.2) to produce acceptable results with the performance check solution.

11.8.3. Acquire SIM data according to Section 6.1.3. Use the same acquisition and mass spectrometer operating conditions previously used to determine the relative response factors (Section 10). Ions characteristic for polychlorinated diphenyl ethers are included in the descriptors listed in Table 6. Their presence is used to monitor their interference during the characterization of PCDFs.

11.8.4. Identification Criteria

For a gas chromatographic peak to be identified as a PCDD or PCDF, it must meet all of the following criteria:

11.8.4.1. Retention Times

11.8.4.1.1. For 2,3,7,8-substituted congeners, which have an isotopically labeled internal or recovery standard present in the sample extract, the retention time (at maximum peak height) of the sample components (i.e., the two ions used for quantitation purposes listed in Table 6) must be within -1 and +3 seconds of the retention time of the peak for the isotopically labeled internal or recovery standard at m/z corresponding to the first characteristic ion (of the set of two; Table 6) to obtain a positive identification of these nine 2,3,7,8-substituted PCDDs/PCDFs and OCDD.

11.8.4.1.2. For 2,3,7,8-substituted compounds that do not have an isotopically labeled internal standard present in the sample extract, the relative retention time (relative to the appropriate internal standard) must fall within 0.005 relative retention time units of the relative retention times measured in the daily routine calibration. Identification of OCDF is based on its retention time relative to 13C-OCDD as determined from the daily routine calibration results.

11.8.4.1.3. For non-2,3,7,8-substituted compounds (tetra through octa; totaling 119 congeners), the retention time must be within the corresponding homologous retention time windows established by analyzing the column performance check solution.

11.8.4.1.4. The ion current responses for both ions used for quantitative purposes (e.g., for TCDDs: m/z 319.8965 and 321.8936) must reach a maximum simultaneously (± 2 seconds).

11.8.4.1.5. The ion current responses for both ions used for the labeled standards (e.g., for 13C-TCDD: m/z 331.9368 and m/z 333.9339) must reach a maximum simultaneously (± 2 seconds).

11.8.4.2. Ion Abundance Ratios

11.8.4.2.1. The integrated ion current for the two ions used for quantitation purposes must have a ratio between the lower and upper limits established for the homologous series to which the peak is assigned. See Table 9.

11.8.4.3. Signal-To-Noise Ratio

11.8.4.3.1. All ion current intensities must be >2.5 times noise level for positive identification of the PCDD/PCDF compound or a group of coeluting isomers. Figure 7 describes the procedure to be followed for the determination of the S/N.

11.8.4.4. Polychlorinated Diphenyl Ether Interferences

11.8.4.4.1. In addition to the above criteria, the identification of a GC peak as a PCDF can only be made if no signal having a S/N >2.5 is detected, at the same retention time (± 2 seconds), in the corresponding polychlorinated diphenyl ether (PCDPE, Table 6) channel.

12. DATA ANALYSIS AND CALCULATIONS

12.1. For gas chromatographic peaks that have met the criteria outlined in Section 11.5.4 calculate the concentration of the PCDD or PCDF compounds using the formula:

$$C_x = \frac{A_x \times Q_{is}}{A_{is} \times W \times RRF\,(n)}$$

Where:

C_x = concentration of unlabeled PCDD/PCDF congeners (or group of coeluting isomers within an homologous series) usually in pg/g or pg/L,

Ax = sum of the integrated ion abundances of the quantitation ions (Table 6) for the unlabeled PCDD/PCDFs,

Ais = sum of the integrated ion abundances of the quantitation ions (Table 6) for the labeled internal standards,

Qis = quantity, in pg, of the internal standard added to the sample before extraction,

W = sample size in g (if solid) or L (if liquid).

$RRF(n)$ = Calculated mean relative response factor for the analyte

[RRF(n) with n = 1 to 17; Section 10.3.5].

If the analyte is identified as one of the 2,3,7,8-substituted PCDDs or PCDFs, RRF(n) is the value calculated using the equation in Section 10.3.5.1. However, if it is a non-2,3,7,8-substituted congener, the RRF(k) value is the one calculated using the equation in Section 10.3.5.3.2 [RRF(k) with k = 27 to 30].

12.2. Calculate the percent recovery of the nine internal standards measured in the sample extract, using the formula:

$$\text{Internal Standard Percent Recovery} = \frac{A_{is} \times Q_{rs}}{Q_{is} \times A_{rs} \times RRF\,(m)} \times 100$$

Where:

Ais = sum of the integrated ion abundances of the quantitation ions (Table 6) for the labeled internal standard,

Ars = sum of the integrated ion abundances of the quantitation ions (Table 6) for the labeled recovery standard; the selection of the recovery standard depends on the type of congeners (see Table 5, footnotes),

Qis = Quantity, in pg, of the internal standard added to the sample before extraction,

Qrs = Quantity, in pg, of the recovery standard added to the cleaned-up sample residue before HRGC/HRMS analysis, and

RRF(m) = calculated mean relative response factor for the labeled internal standard relative to the appropriate (see Table 5, footnotes) recovery standard. This represents the mean obtained in Section 10.3.5.4 [RRF(m) with m = 18 to 26].

12.3. If the concentration in the final extract of any of the fifteen 2,3,7,8-substituted PCDD/PCDF compounds (Table 3) exceeds the upper method calibration limit (MCL) for that compound listed in Table 1, the linear range of response versus concentration may have been exceeded. In such cases, the following corrective actions will be undertaken:

- If the signal for the analyte has saturated the detector, a single dilution and reanalysis of the extract will be made in an attempt to bring the signal within the range of the detector. If the measured concentration of the analyte is still above the MCL, the reported concentration for the analyte will be qualified appropriately.

- If the signal for the analyte is above the MCL but does not saturate the detector, the concentration will be reported and qualified appropriately.

In either case, **with the approval of the client**, the sample may be re-extracted and/or re-analyzed with one or more of the following adjustments made to the analytical procedure in order to provide a concentration which meets client-specific data quality objectives.

- Extraction and analysis of a one tenth aliquot. This is appropriate if it will provide analyte concentration within the MCL and will also provide a representative sample aliquot.
- Extraction of an aliquot large enough to be representative with an increased concentration of internal standard and surrogate spike components added prior to the extraction. The extract is then diluted either prior to or after the cleanup procedures.
- Dilution of the original extract. Internal standard components are re-spiked at an appropriate level prior to analysis. In this case, the internal standard recoveries are taken from the original analysis.

For the other congeners (including OCDD and OCDF), however, report the measured concentration and indicate that the value exceeds the upper calibration standard.

12.4. The total concentration for each homologous series of PCDD and PCDF is calculated by summing up the concentrations of all positively identified isomers of each homologous series. Therefore, the total should also include the 2,3,7,8-substituted congeners. The total number of GC signals included in the homologous total concentration value may be specified in the report.

12.5. Sample-Specific Estimated Detection Limit

The sample-specific estimated detection limit (EDL) is the concentration of a given analyte required to produce a signal with a peak height of at least 2.5 times the background signal level. An EDL is calculated for each 2,3,7,8-substituted congener that is not identified, regardless of whether or not other non-2,3,7,8-substituted isomers are present. Two methods of calculation can be used, as follows, depending on the type of response produced during the analysis of a particular sample.

12.5.1. Samples giving a response for both quantitation ions (Tables 6 and 9) that is less than 2.5 times the background level.

12.5.1.1. Use the expression for EDL (specific 2,3,7,8-substituted PCDD/PCDF) below to calculate an EDL for each absent 2,3,7,8-substituted PCDD/PCDF (i.e., S/N <2.5). The background level is determined by measuring the range of the noise (peak to peak) for the two quantitation ions (Table 6) of a particular 2,3,7,8-substituted isomer within an homologous series, in the region of the SICP trace corresponding to the elution of the internal standard (if the congener possesses an internal standard) or in the region of the SICP where the congener is expected to elute by comparison with the routine calibration data (for those congeners that do not have a 13C-labeled standard), multiplying that noise height by 2.5, and relating the product to an estimated concentration that would produce that product height.

NOTE: The quantitation ions for both the unlabeled PCDDs/PCDFs and their internal standard must be consistently paired (using either both lighter mass ions or both heavier mass ions).

Use the formula:

$$EDL_{Specific\,2,3,7,8-subst.PCDD\,/\,PCDF} = \frac{2.5 \times H_x \times Q_{is}}{H_{is} \times W \times RRF(n)}$$

$$\text{EDL(specific 2,3,7,8-subst. PCDD/PCDF)} = \frac{2.5 \text{ x } H_x \text{ x } Q_{is}}{H_{is} \text{ x W x RRF(n)}}$$

Where:

EDL = estimated detection limit for homologous 2,3,7,8-substituted PCDDs/PCDFs.

H_x = height of the average noise for one of the quantitation ions (Table 6) for the unlabeled PCDDs/PCDFs.

H_{is} = height of one of the quantitation ions (Table 6) for the labeled internal standards.

W, RRF (n), and Qis retain the same meanings as defined in Section 12.1

12.5.2. Samples characterized by a response above the background level with a S/N of at least 2.5 for at least one of the quantitation ions (Tables 6 and 9).

12.5.2.1. When the response of a signal having the same retention times as a 2,3,7,8-substituted congener has a S/N in excess of 2.5 and does not meet any of the other qualitative identification criteria listed in Section 11.5.4, calculate the "Estimated Maximum Possible Concentration" (EMPC) according to the expression shown in Section 12.1, except that Ax in Section 12.1 should represent the sum of the area under the smaller peak and of the other peak area calculated using the theoretical chlorine isotope ratio. Alternatively, an EDL can be calculated using the above formula and the height of one of the ions as appropriate.

12.6. The relative percent difference (RPD) is calculated as follows:

$$RPD = \frac{|S_1 - S_2|}{(S_1 + S_2)/2} \times 100$$

S_1 and S_2 represent sample and duplicate sample results.

12.7. The 2,3,7,8-TCDD toxic equivalents (TEQ) of PCDDs and PCDFs present in the sample are calculated at the data user's request. This method assigns a 2,3,7,8-TCDD toxicity equivalency factor (TEF) to each of the seventeen 2,3,7,8-substituted PCDDs and PCDFs (Table 11). The 2,3,7,8-TCDD equivalent of the PCDDs and PCDFs present in the sample is calculated by summing the TEF times their concentration for each of the compounds or groups of compounds listed in Table 11.

12.7.1. Two-GC Column TEF Determination

12.7.1.1. The concentration of 2,3,7,8-TCDD (see note below), is calculated from the analysis of the sample extract on the 60m DB-5 fused silica capillary column. The chromatographic separation of this isomer must be < 25% valley.

12.7.1.2. For samples that have a presumptive positive result for 2,3,7,8-TCDF on the DB-5 column, the extract is reanalyzed on a 30m DB-225 fused silica column. The GC/MS conditions are altered so that only the first descriptor (Table 6) is used. The reported concentration for 2,3,7,8-TCDF is then the result calculated from the DB-225 analysis. The chromatographic separation between 2,3,7,8-TCDF and any other unlabeled TCDF isomers must be < 25% valley using the column performance check solution for the DB-225 column. Concentration calculations are performed as in Section 12.1 through 12.6.

12.7.1.3. A DB-225 column can be used in the quantitative analysis of 2,3,7,8-TCDF and 2,3,7,8-TCDD analytes. Since the DB-225 cannot resolve 2,3,7,8-TCDD any positively identified 2,3,7,8-TCDD which exceeds the reporting limit shall be confirmed on a DB-5 column.

12.7.1.4. For a gas chromatographic peak to be identified as a 2,3,7,8-substituted PCDD/PCDF congener, it must meet the ion abundance (Section 11.5.4) and signal-to-noise ratio criteria. In addition, the retention time identification criterion described in Section 11.5.4 applies here for congeners for which a carbon-labeled analog is available in the sample extract. However, the relative retention time (RRT) of the 2,3,7,8-substituted congeners for which no carbon-labeled analogs are available must fall within 0.006 units of the carbon-labeled standard RRT. Experimentally, this is accomplished by using the attributions described in Table 12 and the results from the routine calibration run on the DB-5 column.

13. METHOD PERFORMANCE

It must be documented that all applicable system performance criteria specified were met before analysis of any sample is performed. Table 7 provides recommended GC conditions that can be used to satisfy the required criteria. Figure 4 provides a typical 12-hour analysis sequence. A GC column

performance check is only required at the beginning of each 12-hour period during which samples are analyzed.

13.1. GC Column Performance

13.1.1. Inject 1 or 2 µL of the column performance check solution and acquire selected ion monitoring (SIM) data as described in Section 6.1.3 within a total cycle time of < 1 second.

13.1.2. The chromatographic separation between 2,3,7,8-TCDD and the peaks representing any other TCDD isomers must be resolved with a valley of ≤ 25 percent (Figure 5),

Where:

$$\text{Valley Percent} = (x/y) \times 100$$

x = measured as in Figure 5 from the 2,3,7,8-closest TCDD eluting isomer,

y = the peak height of 2,3,7,8-TCDD

13.1.3. It is the responsibility of the laboratory to verify the conditions suitable for the appropriate resolution of 2,3,7,8-TCDD from all other TCDD isomers. The GC column performance check solution also contains the known first and last PCDD/PCDF eluters under the conditions specified in this protocol. Their retention times are used for qualitative and quantitative purposes. The peak for 2,3,7,8-TCDD must be labeled on the chromatograms. The chromatograms showing the first and last eluters of a homologous series must be included.

13.1.4. The retention times for the switching of SIM ions characteristic of one homologous series to the next higher homologous series must be indicated in the SICP. Accurate switching at the appropriate times is absolutely necessary for accurate monitoring of these compounds.

14. POLLUTION PREVENTION

14.1. No solvents/wastes of any kind or in any amount are to be disposed of in the sinks or evaporated in the hoods.

15. WASTE MANAGEMENT

15.1. Waste management in the procedure must be segregated and disposed according to the waste streams identified in the Corporate Safety Manual

15.2. Samples and other solutions containing high concentrations of toxic materials must be disposed of according to the Corporate Safety Manual.

16. REFERENCES

16.1. SW846, Test Methods for Evaluating Solid Waste, Third edition, Update III. Polychlorinated Dibenzodioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) by high-Resolution Mass Spectrometry September 1994.

16.2. Method 0023A, December 1996.

16.3. Protocol for the Analysis of 2,3,7,8-TCDD by HRGC/HRMS". J. S. Stanley and T. M. Sack, EPA 600/4-86-004.

16.4. "Safety in Academic Chemistry Laboratories", American Chemical Society Publication, Committee on Chemical Safety (3rd Edition, 1979.)

16.5. "Carcinogens - Working with Carcinogens". Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control. National Institute for Occupational Safety and Health. Publication No. 77-206, August 1977.

16.6. "OSHA Safety and Health Standards, General Industry", (29 CFR 1910) Occupational Safety and Health Administration, OSHA 2206 (revised January 1976).

17. MISCELLANEOUS (TABLES, APPENDICES, ETC...)

17.1. Deviations from reference method.

17.1.1. The method specifies that 2 µL injections are used throughout the analysis. If an instrument demonstrates adequate sensitivity and chromatographic resolution, then the analyst may use 1 µL injections for all performance checks, standards, QC samples, and samples.

17.1.2. In Section 2.7 of Method 8290, a retention time window of 0.005 RT units is used to tentatively identify unlabeled PCDD/PCDFs for which there are no

corresponding labeled internal standards. All available labeled internal standards are used; therefore, a retention time window of -1 to +3 seconds is used to identify all compounds. See Section 7.8.4.1 of Method 8290.

17.1.3. Tetradecane instead of nonane is used as the final solvent to increase the stability of extracts and standards. Tetradecane is less volatile than nonane. Loss of analyte as a result of solvent incompatibility is monitored through recovery checks and calibration acceptance criteria.

17.1.4. Extract clean-ups are performed at the discretion of the analyst when interferences are observed. Then, the analyst should select the clean-up procedure appropriate to the interferent.

17.1.5. Section 7.4.6.4 of Method 8290 indicates that extracts should be transferred with hexane, then toluene. Toluene is used to transfer extracts to maintain compound solubility and minimize analyte loss.

17.1.6. Section 7.5.1.2 of Method 8290 specifies that a NaCl solution should be used for partitioning. Instead the laboratory uses laboratory water only. NaCl is used to break up emulsions that may form. An analyst may use NaCl, NaOH, or any other mechanical means to break up an emulsion.

17.1.7. Section 7.5.3 of Method 8290 specifies that hexane is used as a column elution solvent. The laboratory uses cyclohexane to achieve better and more reproducible separation of the target analyte from the interferent.

17.1.8. Carbon columns are packed with silica gel in place of celite. Elution solvents are changed accordingly. (SOP Section 11.4; Method 8290 Section 7.5.3.2).

17.1.9. Modifications from TO-9A method

17.1.9.1. Quartz Fiber Filters are cleaned by Soxhlet extraction with methylene chloride, not baked at 400 degrees C for 5 hours.

17.1.9.2. The PUF material is pre-cleaned with methlyene chloride not acetone. The PUFs are not reused.

17.1.9.3. Samples are extracted with toluene not benzene.

17.1.9.4. Concentration is performed by rotary evaporation not Kuderna-Danish.

17.1.9.5. All cleanup procedures are optional and applied based on the analyst's discretion.

17.1.9.6. The final volume is adjusted to 20 uL in tetradecane.

17.1.9.7. Calibration and quantitation are performed in accordance to this SOP.

17.2. Summary of modifications to SOP from previous revisions

17.2.1. The SOP format was updated.

17.2.2. Modified to include extraction and analysis of ambient air samples collected in filter/PUF material.

17.2.3. Added sample dilution procedure.

17.2.4. Added extraction procedure for crawfish.

17.2.5. Added procedure to eliminate emulsions.

17.3. Tables or figures referenced in body of SOP.

17.3.1. Table 1 - Types of Matrices

17.3.2. Table 2 - Composition of The Sample Fortification and Recovery Standard Solutions.

17.3.3. Table 3 - The Fifteen 2,3,7,8-Substituted PCDD and PCDF Congeners

17.3.4. Table 4 - Isomers of Chlorinated Dioxins and Furans

17.3.5. Table 5 - Concentrations of Calibration Solutions

17.3.6. Table 6 - Ions Monitored for PCDDs/PCDFs

17.3.7. Table 7 - Recommended GC Operating Conditions

17.3.8. Table 8 - Congeners in the GC Performance Evaluation Solution (DB-5)

17.3.9. Table 9 - Theoretical Ion Abundance Ratios and Control Limits

17.3.10. Table 10 - Relative Response Factor Attributes

17.3.11. Table 11 - 2,3,7,8-TCDD Equivalent Factors

17.3.12. Table 12 - TEF: Analyte Relative Retention Time Reference Attributes

17.3.13. Figure 1 - Analysis Flowchart

17.3.14. Figure 2 - Compound Structure

17.3.15. Figure 3 - PFK Resolution Example

17.3.16. Figure 4 - Analysis Scheme

17.3.17. Figure 5 - GC Performance Check Chromatogram on the DB-5 Column

17.3.18. Figure 6 - PFK Peak Profile

17.3.19. Figure 7 - Manual Determination of Signal-to-Noise

17.3.20. Appendix A - Periodic Wipe Test Performance

TABLE 1

Types of Matrices, Sample Sizes and 2,3,7,8-TCDD-Based Method Calibration Limits (Parts per Trillion)

	Water	Soil Sediment Paper Pulp	Fly Ash	Human/ Fish Tissue	Adipose Tissue	Sludges, Fuel Oil	Still-Bottom	Ambient or Source Samples
Lower MCL(a)	0.02	2.0	2.0	2.0	2.0	10	20	40
Upper MCL(a)	4.0	400	400	400	400	2000	4000	8000
Weight (g)	1000	10	10	10	10	2.0	1.0	1 sample
IS Spiking Levels (ng)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	4.0
Final Extract Volume (µL)	20	20	20	20	20	20	20	20

(a) For other congeners, multiply the values by 1 for TCDF, by 2.5 for PeCDD/PeCDF/HxCDD/HxCDF/HpCDD/HpCDF, and by 5 for OCDD/OCDF.

TABLE 2

Composition of the Sample Fortification and Recovery Standard Solutions

Analyte	Sample Fortification Solution Concentration pg/µL; Solvent: Isooctane	Sample Fortification Solution Concentration pg/µL; Solvent: Tetradecane
13C-2,3,7,8-TCDD	10	--
13C-2,3,7,8-TCDF	10	--
13C-1,2,3,4-TCDD	--	100
13C-1,2,3,7,8-PeCDD	10	--
13C-1,2,3,7,8-PeCDF	10	--
13C-1,2,3,6,7,8-HxCDD	25	--
13C-1,2,3,4,7,8-HxCDF	25	--
13C-1,2,3,7,8,9-HxCDD	--	100
13C-1,2,3,4,6,7,8-HpCDD	25	--
13C-1,2,3,4,6,7,8-HpCDF	25	--
13C-OCDD	50	--

TABLE 3

The Seventeen 2,3,7,8-Substituted PCDD and PCDF Congeners

PCDD	PCDF
2,3,7,8-TCDD(*)	2,3,7,8-TCDF(*)
1,2,3,7,8-PeCDD(*)	1,2,3,7,8-PeCDD(*)
1,2,3,6,7,8-HxCDD(*)	2,3,4,7,8-PeCDF
1,2,3,4,7,8-HxCDD	1,2,3,6,7,8-HxCDF
1,2,3,7,8,9-HxCDD(+)	1,2,3,7,8,9-HxCDF
1,2,3,4,6,7,8-HpCDD(*)	1,2,3,4,7,8-HxCDF(*)
1,2,3,4,5,6,7,8-OCDD(*)	2,3,4,6,7,8-HxCDF
	1,2,3,4,6,7,8-HpCDF(*)
	1,2,3,4,7,8,9-HpCDF
	1,2,3,4,5,6,7,8-OCDF

(*)The 13C-labeled analog is used as an internal standard.
(+)The 13C-labeled analog is used as a recovery standard.

TABLE 4

Isomers of Chlorinated Dioxins and Furans as a Function of the Number of Chlorine Atoms

# of Chlorine Atoms	# of Dioxin Isomers	# of 2,3,7,8 Isomers	# of Furan Isomers	# of 2,3,7,8 Isomers
1	2	---	4	---
2	10	---	16	---
3	14	---	28	---
4	22	1	38	1
5	14	1	28	2
6	10	3	16	4
7	2	1	4	2
8	1	1	1	1
Total	**75**	**7**	**135**	**10**

TABLE 5

High Resolution Concentration Calibration Solutions

Compound	Concentration (ng/mL)				
	CS1	CS2	CS3 (VER(6))	CS4	CS5
Native CDDs and CDFs					
2,3,7,8-TCDD	0.5	2	10	40	200
2,3,7,8-TCDF	0.5	2	10	40	200
1,2,3,7,8-PeCDD	2.5	10	50	200	1000
1,2,3,7,8-PeCDF	2.5	10	50	200	1000
2,3,4,7,8-PeCDF	2.5	10	50	200	1000
1,2,3,4,7,8-HxCDD	2.5	10	50	200	1000
1,2,3,6,7,8-HxCDD	2.5	10	50	200	1000
1,2,3,7,8,9-HxCDD	2.5	10	50	200	1000
1,2,3,4,7,8-HxCDF	2.5	10	50	200	1000
1,2,3,6,7,8-HxCDF	2.5	10	50	200	1000
1,2,3,7,8,9-HxCDF	2.5	10	50	200	1000
2,3,4,6,7,8-HxCDF	2.5	10	50	200	1000
1,2,3,4,6,7,8-HpCDD	2.5	10	50	200	1000
1,2,3,4,6,7,8-HpCDF	2.5	10	50	200	1000
1,2,3,4,7,8,9-HpCDF	2.5	10	50	200	1000
OCDD	5.0	20	100	400	2000
OCDF	5.0	20	100	400	2000
Labeled CDDs and CDFs					
$^{13}C_{12}$-2,3,7,8-TCDD	100	100	100	100	100
$^{13}C_{12}$-2,3,7,8-TCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8-PeCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8-PeCDF	100	100	100	100	100
$^{13}C_{12}$-2,3,4,7,8-PeCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	100	100	100	100	100
$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	100	100	100	100	100
$^{13}C_{12}$-OCDD	200	200	200	200	200
Cleanup Standard/ FS					
$^{37}Cl_4$-2,3,7,8-TCDD	0.5	2	10	40	200
Recovery Standards					
$^{13}C_{12}$-1,2,3,4-TCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	100	100	100	100	100

TABLE 6*

Ions Monitored for HRGC/HRMS Analysis of PCDDs/PCDFs

Descriptor	Accurate[(a)] Mass	Ion ID	Elemental Composition	Analyte
1	303.9016	M	$C_{12}H_4{}^{35}Cl_4O$	TCDF
	305.8987	M+2	$C_{12}H_4{}^{35}Cl_3{}^{37}ClO$	TCDF
	315.9419	M	$^{13}C_{12}H_4{}^{35}Cl_4O$	TCDF (S)
	317.9389	M+2	$^{13}C_{12}H_4{}^{35}Cl_3{}^{37}ClO$	TCDF (S)
	319.8965	M	$C_{12}H_4{}^{35}Cl_4O_2$	TCDD
	321.8936	M+2	$C_{12}H_4{}^{35}Cl_3{}^{37}ClO_2$	TCDD
	331.9368	M	$^{13}C_{12}H_4{}^{35}Cl_4O_2$	TCDD (S)
	333.9338	M+2	$^{13}C_{12}H_4{}^{35}Cl_3{}^{37}ClO_2$	TCDD (S)
	375.8364	M+2	$C_{12}H_4{}^{35}Cl_5{}^{37}ClO$	HxCDPE
	[354.9792]	LOCK	C_9F_{13}	PFK
2	339.8597	M+2	$C_{12}H_3{}^{35}Cl_4{}^{37}ClO$	PeCDF
	341.8567	M+4	$C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O$	PeCDF
	351.9000	M+2	$^{13}C_{12}H_3{}^{35}Cl_4{}^{37}ClO$	PeCDF (S)
	353.8970	M+4	$^{13}C_{12}H_3{}^{35}Cl_4{}^{37}ClO$	PeCDF (S)
	355.8546	M+2	$C_{12}H_3{}^{35}Cl_4{}^{37}ClO_2$	PeCDD
	357.8516	M+4	$C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O_2$	PeCDD
	367.8949	M+2	$^{13}C_{12}H3{}^{35}Cl_4{}^{37}ClO_2$	PeCDD (S)
	369.8919	M+4	$^{13}C_{12}H3{}^{35}Cl_3{}^{37}Cl_2O_2$	PeCDD (S)
	409.7974	M+2	$C_{12}H_3{}^{35}Cl_6{}^{37}ClO$	HpCDPE
	[354.9792]	LOCK	C_9F_{13}	PFK
3	373.8208	M+2	$C_{12}H_2{}^{35}Cl_5{}^{37}ClO$	HxCDF
	375.8178	M+4	$C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O$	HxCDF
	383.8639	M	$^{13}C_{12}H_2{}^{35}Cl_6O$	HxCDF (S)
	385.8610	M+2	$^{13}C_{12}H_2{}^{35}Cl_5{}^{37}ClO$	HxCDF (S)
	389.8156	M+2	$C_{12}H_2{}^{35}Cl_5{}^{37}ClO_2$	HxCDD
	391.8127	M+4	$C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O_2$	HxCDD
	401.8559	M+2	$^{13}C_{12}H_2{}^{35}Cl_5{}^{37}ClO_2$	HxCDD (S)
	403.8529	M+4	$^{13}C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O_2$	HxCDD (S)

TABLE 6 (cont.)*

Ions Monitored for HRGC/HRMS Analysis of PCDDs/PCDFs

Descriptor	Accurate[(a)] Mass	Ion ID	Elemental Composition	Analyte
4	407.7818	M+2	$C_{12}H^{35}Cl_6{}^{37}ClO$	HpCDF
	409.7788	M+4	$C_{12}H^{35}Cl_5{}^{37}Cl_2O$	HpCDF
	417.8250	M	$^{13}C_{12}H^{35}Cl_7O$	HpCDF (S)
	419.8220	M+2	$^{13}C_{12}H^{35}Cl_6{}^{37}ClO$	HpCDF
	423.7767	M+2	$C_{12}H^{35}Cl_6{}^{37}ClO_2$	HpCDD
	425.7737	M+4	$C_{12}H^{35}Cl_5{}^{37}Cl_2O_2$	HpCDD
	435.8169	M+2	$^{13}C_{12}H^{35}Cl_6{}^{37}ClO_2$	HpCDD (S)
	437.8140	M+4	$^{13}C_{12}H^{35}Cl_5{}^{37}CL_2O_2$	HpCDD (S)
	479.7165	M+4	$C_{12}H^{35}CL_7{}^{37}Cl_2O$	NCDPE
	[430.9728]	LOCK	C_9F_{17}	PFK
5	441.7428	M+2	$C_{12}{}^{35}Cl_7{}^{37}ClO$	OCDF
	443.7399	M+4	$C_{12}{}^{35}Cl_6{}^{37}Cl_2O$	OCDF
	457.7377	M+2	$C_{12}{}^{35}Cl_7{}^{37}ClO_2$	OCDD
	459.7348	M+4	$C_{12}{}^{35}Cl_6{}^{37}Cl_2O_2$	OCDD
	469.7780	M+2	$^{13}C1_2{}^{35}Cl_7{}^{37}ClO_2$	OCDD (S)
	471.7750	M+4	$^{13}C1_2{}^{35}Cl_6{}^{37}Cl_2O_2$	OCDD (S)
	513.6775	M+4	$^{13}C1_2{}^{35}Cl_8{}^{37}Cl_2O$	DCDPE
	[442.9728]	LOCK	$C_{10}F_{17}$	PFK

[(a)] The following nuclidic masses were used:

H =	1.007825	O =	15.994915
C =	12.000000	^{35}Cl =	34.968853
^{13}C =	13.003355	^{37}Cl =	36.965903
F =	18.9984		

S = Internal/recovery standard

*The homologous groups for functions 1-3 do not use the same lockmass as described in Table 6. They use masses 316.9824, 366.9792, and 380.9760, respectively.

TABLE 7

Recommended GC Operating Conditions

The GC Operating Conditions (Temperatures (°C), and Times (minutes))
Are as Follows:

Injector Temperature: 280°C

Interface Temperature: 280°C

Initial Temperature and Time: 190°C / 1 Minute

Temperature Program: 190°C, increasing at a rate of 4°C per minute up to 240°C, and maintaining at this temperature until the last of the tetra- group has eluted from the column. (The total time required for this is approximately 25 minutes, depending on the length of the column). The maintained temperature of 240°C is then increased to 320°C at the rate of 20°C per minute and held at this level until the last compound (octa-group) has eluted from the column.

TABLE 8

PCDD and PCDF Congeners Present in the GC Performance Evaluation Solution and Used for Defining the Homologous GC Retention Time Windows on a 60-M DB-5 Column[b]

# of Chlorine Atoms	PCDD Positional Isomer		PCDF Positional Isomer	
	Early Eluter	Late Eluter	Early Eluter	Late Eluter
4[a]	1,3,6,8	1,2,8,9	1,3,6,8	1,2,8,9
5	1,2,4,6,8/1,2,4,7,9	1,2,3,8,9	1,3,4,6,8	1,2,3,8,9
6	1,2,3,4,6,8	1,2,3,4,6,7	1,2,3,4,6,8	1,2,3,4,8,9
7	1,2,3,4,6,7,8	1,2,3,4,6,7,9	1,2,3,4,6,7,8	1,2,3,4,6,7,9
8	1,2,3,4,6,7,8,9		1,2,3,4,6,7,8,9	

[a] In addition to these two PCDD isomers, the 1,2,3,4-, 1,2,3,7-, 1,2,3,8-, 2,3,7,8-, $^{13}C_{12}$-2,3,7,8-, and 1,2,3,9-TCDD isomers must also be present.

(b) The PCDF Congeners present in GC the Performance Evaluation Solution for the 30 m DB-225 column include:

- 1,2,3,9-TCDF
- 2,3,7,8-TCDF
- 2,3,4,7-TCDF
- $^{13}C_{12}$-2,3,7,8-TCDF

Column performance criteria is met when the percent valleys between the 2,3,7,8-TCDF analyte and the closest eluting isomers are $\leq$ 25%.

TABLE 9

Theoretical Ion Abundance Ratios and Their Control Limits for PCDDs and PCDFs

# of Chlorine Atoms	Ion Type	Theoretical Ratio	Control Limits	
			Lower	Upper
4	M / M+2	0.77	0.65	0.89
5	M+2 / M+4	1.55	1.32	1.78
6	M+2 / M+4	1.24	1.05	1.43
6[(a)]	M / M+2	0.51	0.43	0.59
7[(b)]	M / M+2	0.44	0.37	0.51
7	M+2 / M+4	1.04	0.88	1.20
8	M+2 / M+4	0.89	0.76	1.02

(a) Used only for ^{13}C-HxCDF (IS)
(b) Used only for ^{13}C-HpCDF (IS)

TABLE 10

Relative Response Factor [RRF (number)] Attributes

Number	Specific Congener Name
1	2,3,7,8-TCDD (and total TCDDs)
2	2,3,7,8-TCDF (and total TCDFs)
3	1,2,3,7,8-PeCDD (and total PeCDDs)
4	1,2,3,7,8-PeCDF
5	2,3,4,7,8-PeCDF
6	1,2,3,4,7,8-HxCDD
7	1,2,3,6,7,8-HxCDD
8	1,2,3,7,8,9-HxCDD
9	1,2,3,4,7,8-HxCDF
10	1,2,3,6,7,8-HxCDF
11	1,2,3,7,8,9-HxCDF
12	2,3,4,6,7,8-HxCDF
13	1,2,3,4,6,7,8-HpCDD (and total HpCDDs)
14	1,2,3,4,6,7,8-HpCDF
15	1,2,3,4,7,8,9-HpCDF
16	OCDD
17	OCDF
18	$^{13}C_{12}$-2,3,7,8-TCDD
19	$^{13}C_{12}$-2,3,7,8-TCDF
20	$^{13}C_{12}$-1,2,3,7,8-PeCDD
21	$^{13}C_{12}$-1,2,3,7,8-PeCDF
22	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD
23	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF
24	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD
25	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF
26	$^{13}C_{12}$-OCDD
27	Total PeCDFs
28	Total HxCDFs
29	Total HxCDDs
30	Total HpCDFs

TABLE 11

2,3,7,8-TCDD Equivalent Factors (TEFs) for the Polychlorinated Dibenzodioxins and Dibenzofurans

Number	Compound (s)	TEF
1	2,3,7,8-TCDD	1.00
2	1,2,3,7,8-PeCDD	0.50
3	1,2,3,6,7,8-HxCDD	0.10
4	1,2,3,7,8,9-HxCDD	0.10
5	1,2,3,4,7,8-HxCDD	0.10
6	1,2,3,4,6,7,8-HpCDD	0.01
7	1,2,3,4,6,7,8,9-OCDD	0.001
8	2,3,7,8-TCDF	0.1
9	1,2,3,7,8-PeCDF	0.05
10	2,3,4,7,8-PeCDF	0.5
11	1,2,3,6,7,8-HxCDF	0.1
12	1,2,3,7,8,9-HxCDF	0.1
13	1,2,3,4,7,8-HxCDF	0.1
14	2,3,4,6,7,8-HxCDF	0.1
15	1,2,3,4,6,7,8-HpCDF	0.01
16	1,2,3,4,7,8,9-HpCDF	0.01
17	1,2,3,4,6,7,8,9-OCDF	0.001

TABLE 12

Toxicity Equivalency Factor:
Analyte Relative Retention Time Reference Attributes

Analyte	Analyte RRT Reference[a]
1,2,3,4,7,8-HxCDD	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD
1,2,3,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF
1,2,3,7,8,9-HxCDF	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF
2,3,4,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF

(a) The retention time of 1,2,3,4,7,8-PeCDF on the DB-5 column is measured relative to $^{13}C_{12}$-1,3,7,8-PeCDF and the retention time of 1,2,3,4,7,8,9-HpCDF relative to $^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF.

FIGURE 1

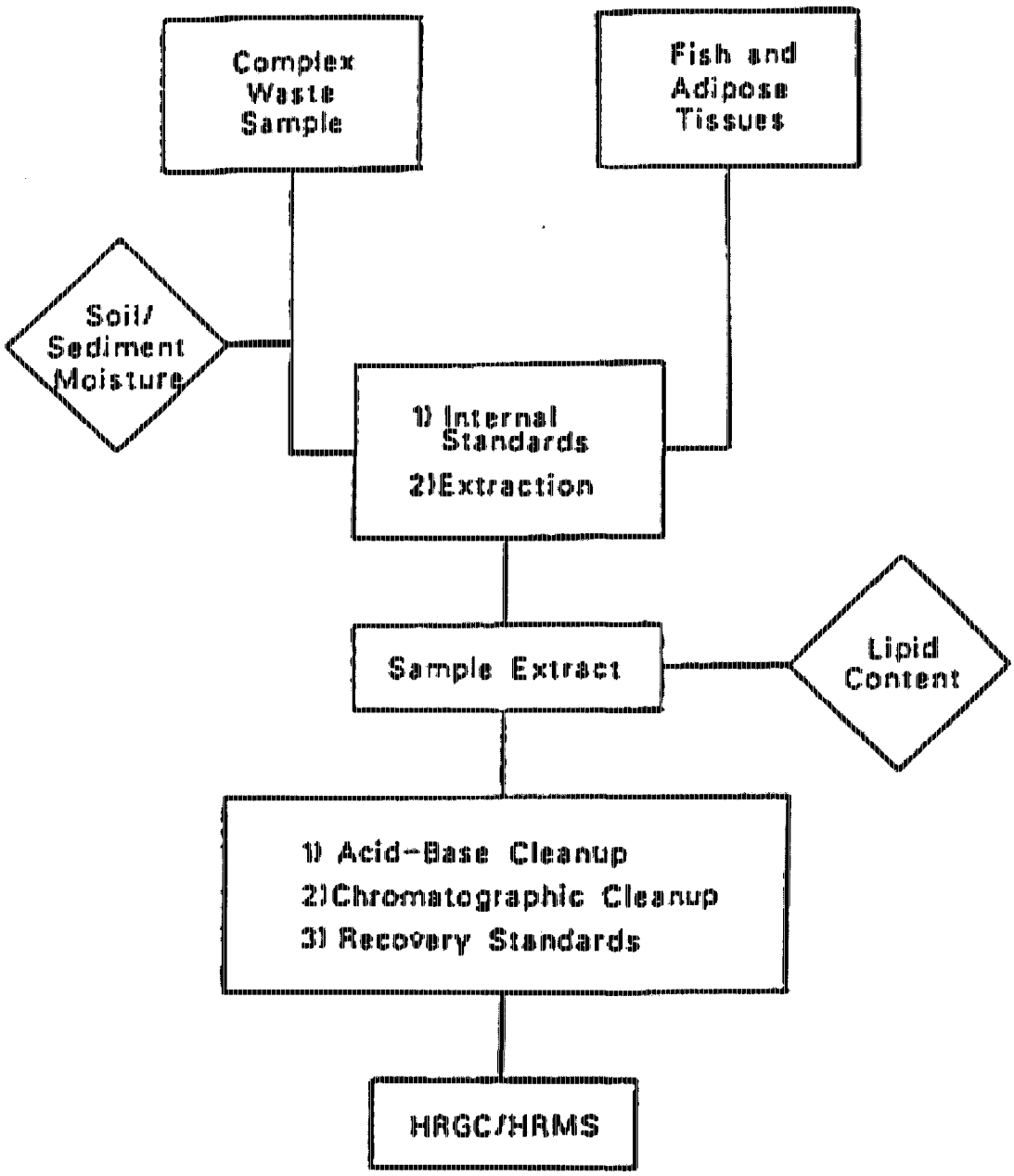
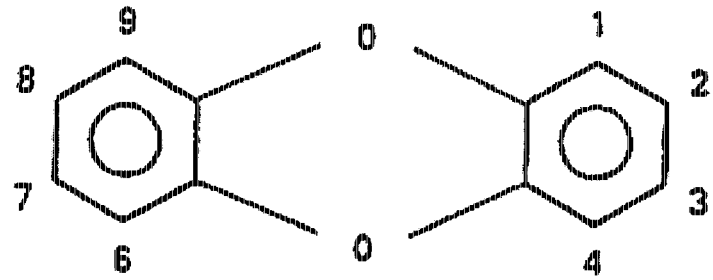
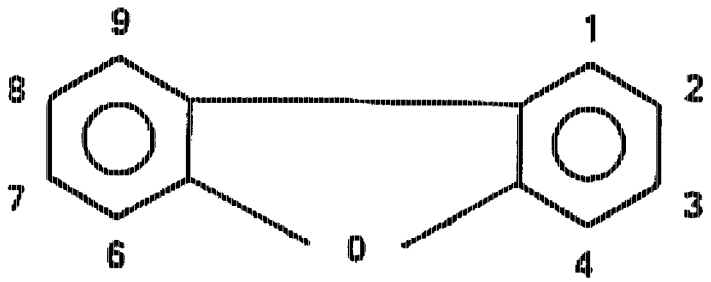

Complex Waste Sample
Fish and Adipose Tissues
Soil/ Sediment Moisture
1) Internal Standards
2)Extraction
Sample Extract
Lipid Content
1) Acid-Base Cleanup
2)Chromatographic Cleanup
3) Recovery Standards
HRGC/HRMS

FIGURE 2



Dibenzodioxin



Dibenzofuran

FIGURE 3

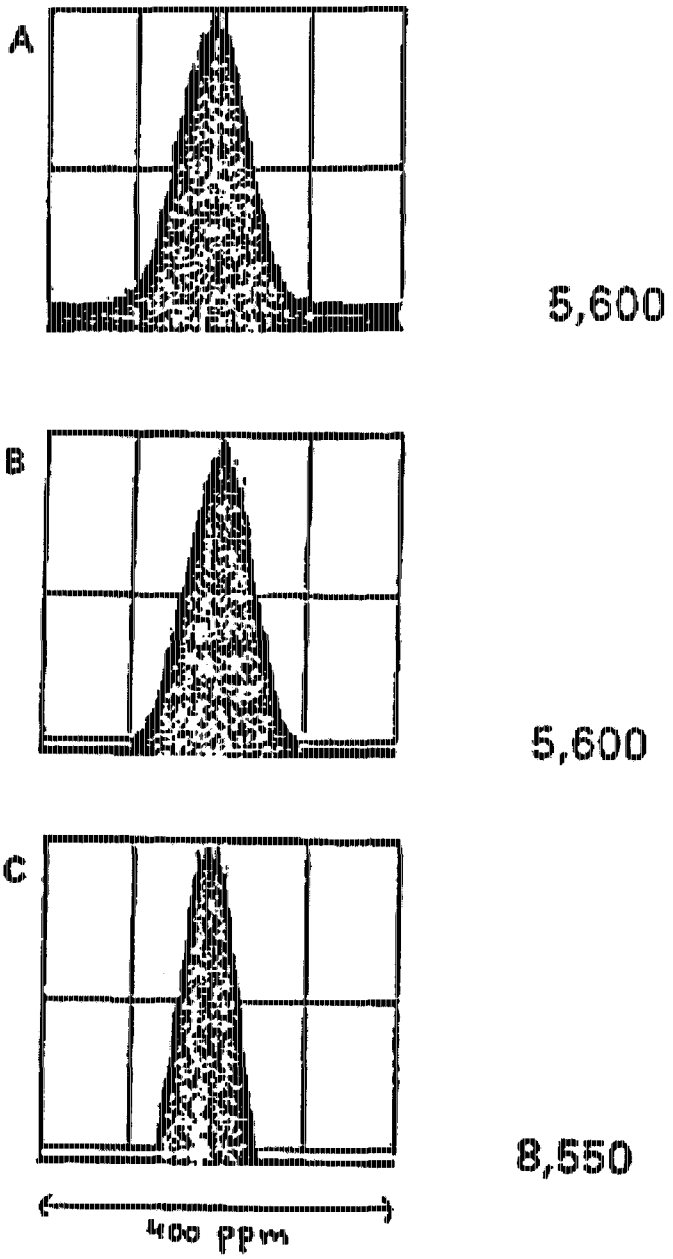


FIGURE 4
Analytical Procedure

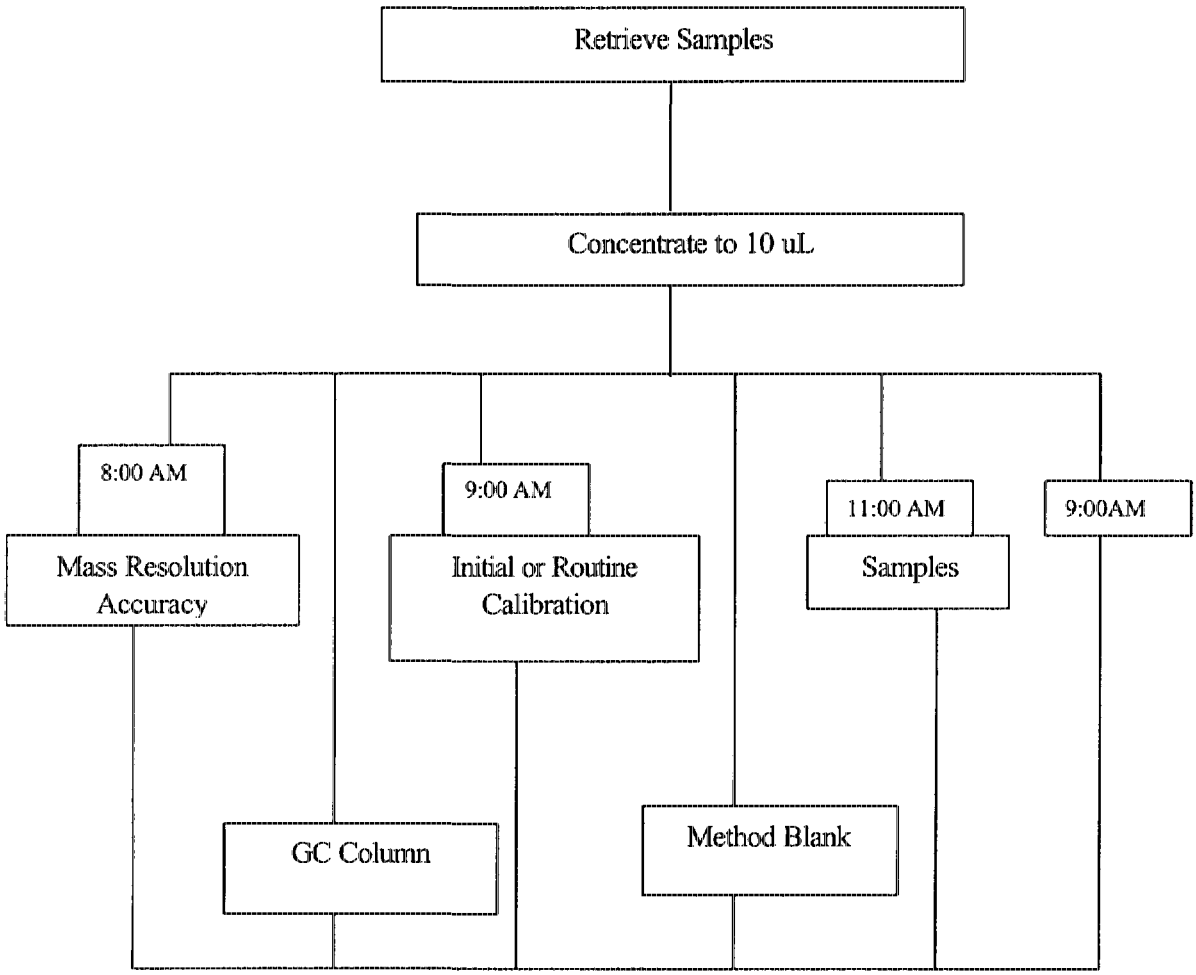
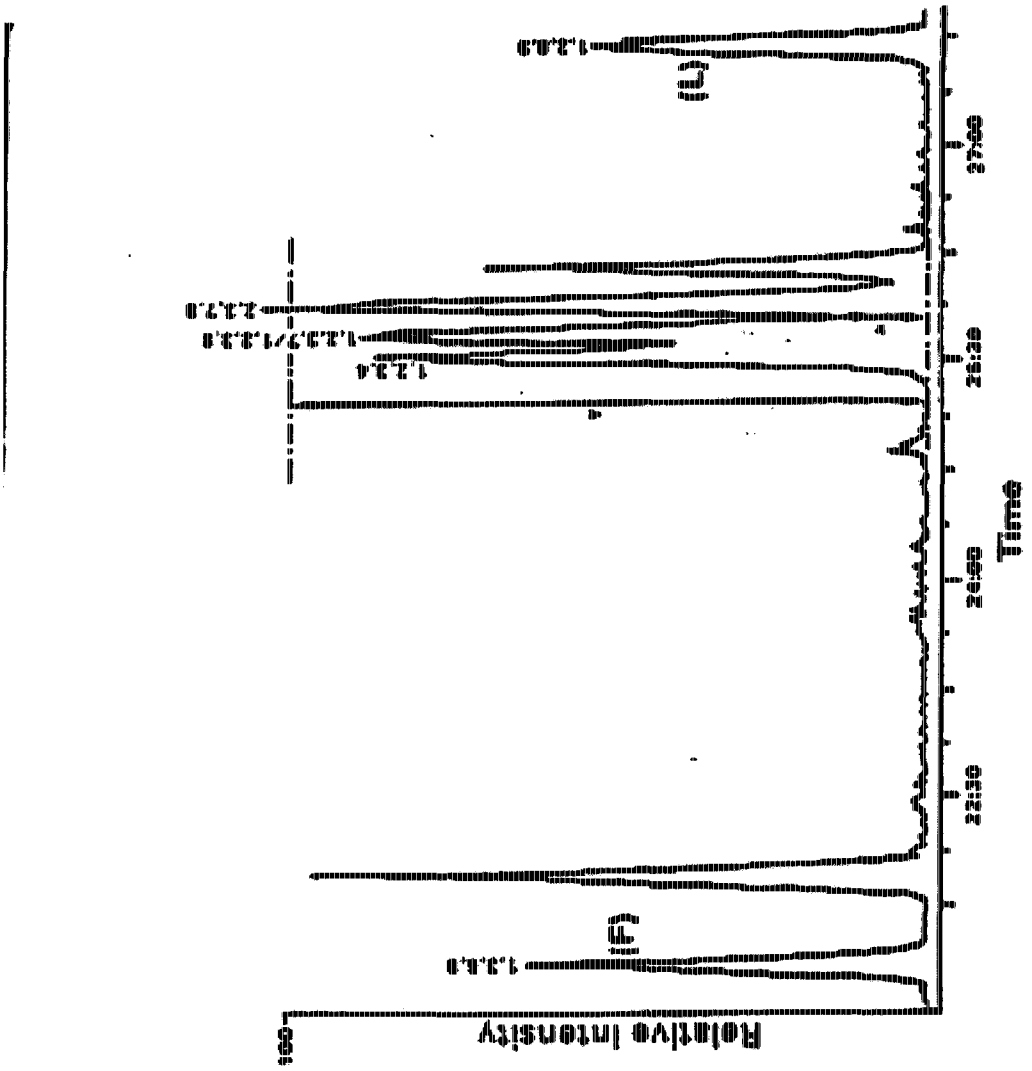
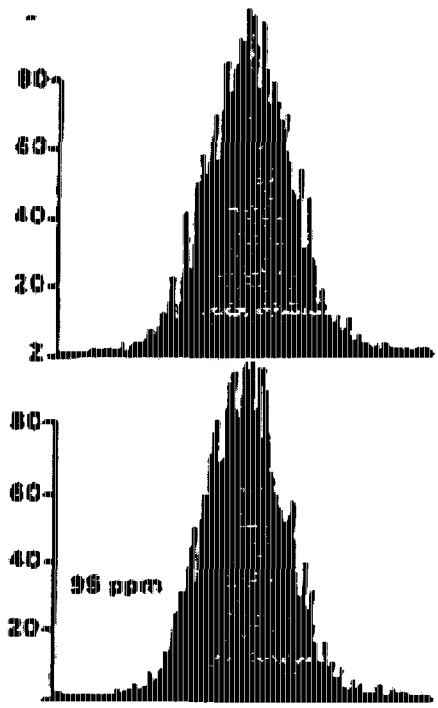


FIGURE 5



Selected ion current profile for m/z 322 (TCDDs) produced by MS analysis of the GC performance check solution on a 60 m DB-5 fused silica capillary column under the conditions listed in Section 7.6.

FIGURE 6



Ref. mass 304.9824 Peak top
Span. 200 ppm

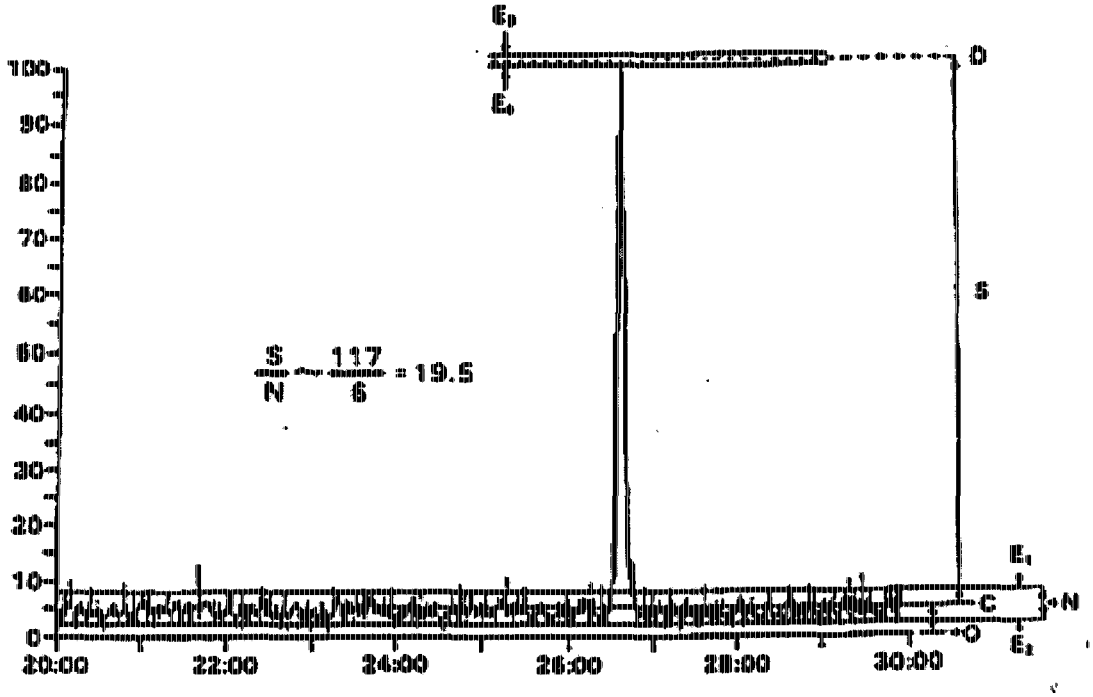
System file name	YVE8150
Data file name	A:062567
Resolution	10000
Group number	1
Ionization mode	EI+
Switching	VOLTAGE
Ref. masses	304.9824
	380.9760

M/ΔM=10,500

Channel B 380.9760 Lock mass
Span 200 ppm

Peak profiles representing two PFK reference ions at m/z 305 and 381. The resolution of the high-mass signal is 95 ppm at 5 percent of the peak height; this corresponds to a resolving power M/ΔM of 10,500 (10 percent valley definition).

FIGURE 7



Manual determination of S/N.

The peak height (S) is measured between the mean noise (lines C and D). These mean signal values are obtained by tracing the line between the baseline average noise extremes, E1 and E2, and between the apex average noise extremes, E3 and E4, at the apex of the signal.

NOTE: It is imperative that the instrument interface amplifier electronic zero offset be set high enough so that negative going baseline noise is recorded.

APPENDIX A

This procedure is designed for the periodic evaluation of potential contamination by 2,3,7,8-substituted PCDD/PCDF congeners of the working areas inside the laboratory.

PERFORMING WIPE TEST

Perform the wipe tests on surface areas of two inches by one foot with laboratory wipers saturated with distilled-in-glass acetone using a pair of clean stainless steel forceps. Use one wiper for each of the designated areas. Combine the wipers to one composite sample in an extraction jar containing 200 mL distilled-in-glass hexane. Place an equal number of unused wipers in 200 mL hexane and use this as a control.

SAMPLE PREPARATION

Close the jar containing the wipers and 200 mL hexane and extract for 20 minutes using a wrist-action shaker. Use an appropriate means to reduce the volume to approximately 1.0 mL. Put through an alumina column to clean up potential interfering compounds. Add appropriate amount of recovery standard.

EXTRACT ANALYSIS

Concentrate the contents of the vial to a final volume of 20 µL (either in a minivial or in a capillary tube). Inject 2 µL of each extract (wipe and control) onto a capillary column and analyze for 2,3,7,8-substituted PCDDs/PCDFs as specified in the analytical method Section 11 (this exhibit).
Perform calculations according to Section 12 (this exhibit).

REPORTING FORMAT

Report the presence of 2,3,7,8-substituted PCDDs and PCDFs as a quantity (pg or ng) per wipe test experiment (WTE). Under the conditions outlined in this analytical protocol, a lower limit of calibration of 25 pg/WTE is expected for 2,3,7,8-TCDD. A positive response for the blank (control) is defined as a signal in the TCDD retention time window at any of the masses monitored which is equivalent to or above 8 pg of 2,3,7,8-TCDD per WTE. For other congeners, use the multiplication factors listed in Table 1, footnote (a) (e.g., for OCDD, the lower MCL is 25 x 5 = 125 pg/WTE and the positive response for the blank would be 8 x 5 = 40 pg). Also, report the recoveries
of the internal standards during the simplified cleanup procedure.

FREQUENCY OF WIPE TESTS

Wipe tests should be performed when there is evidence of contamination in the method blanks.

CORRECTIVE ACTION

An upper limit of 25 pg per TCDD isomer and per wipe test experiment is allowed. (Use multiplication factors listed in footnote (a) from Table 1 for other congeners.) This value corresponds to the lower calibration limit of the analytical method. Steps to correct the contamination must be taken whenever these levels are exceeded. To that effect, first vacuum the working places (hoods, benches, sink) using a vacuum cleaner equipped with a high-efficiency particulate absorbent (HEPA) filter and then wash with a detergent. A new set of wipes should be analyzed before anyone is allowed to work in the dioxin area of the laboratory.

The test results and the decontamination procedure must be reviewed with EH&S.